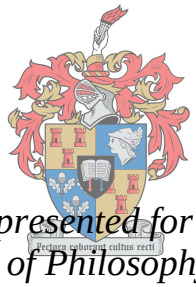
Evaluating the effects of changing global climate on amphibian functional groups of southern Africa: an ecophysiology modelling approach

by

Mohlamatsane McDonald Mokhatla



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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Mohlamatsane Mokhatla

Date: December 2018

Abstract

Global climate change is suggested to be one of the leading threats to anuran diversity by the end of the century. This is largely because, compared to other vertebrates, anurans have characteristically small distribution ranges and limited dispersal abilities, making them particularly vulnerable to climate change. To predict how climatic changes may impact species, biologists have used correlative-based species distribution models (SDMs). However, correlative-based SDMs have been criticised for their simplistic approach of correlating known distributions with climatic variables, and thus not accounting for other process-based variables that determine distributions such as physiology, performance and morphology.

Using a multi-disciplinary approach, the aim of this study is to synergise data from multiple lines of evidence and incorporate them into SDMs to aid in accurately predicting the responses of southern African amphibians with different life-history traits to climate change. In chapter two, I used correlative-based SDMs to determine the impact of past (LGM: Last Glacial Maximum, HGM: Holocene Glacial Minimum) and future (2080) climate change on species distribution ranges of 37, narrowly distributed Cape Floristic Region (CFR) anuran fauna. I found that the biotic velocity at which the CFR anuran community is expected to shift North and East far exceeds historical rates. These models further suggest that the CFR anuran community has already lost ≈ 56% of suitable climate space since the LGM and this is expected to accelerate under future emission scenarios.

Chapter three assessed the impacts of climate change on the distribution ranges of three widely distributed anuran species with different ecological specialisations: *Xenopus laevis*, *Amietia delalandii* and *Sclerophrys capensis*. Although all three species have lost suitable climate since the LGM, the models further suggest that *A. delalandii* is expected to gain suitable climate space by 2080, at least under two GCMs namely, CCSM (25%) and MPI-ESM (7%), while *S. capensis* and *X. laevis* are expected to lose suitable climate space by 2080.

In chapter four, I examined how changes in ambient temperature (T_a) and body mass (M_b) affect body temperature (T_b), standard metabolic rates (SMR) and evaporative water loss (EWL). I found that T_b, whole-animal EWL and whole-animal SMR increased with an increase in temperature. Body temperature increased with an increase in M_b in *A. delalandii* and *S. capensis* but not in *X. laevis*. Whole-animal SMR increased with an increase in M_b in *S. capensis* only.

Chapter five examined the impact of temperature on i) burst swimming and hopping (velocity and acceleration) and hoppping endurance (distance and time) in the same three frog species. Here, I show that temperature changes affect thermal reaction norms only in terrestrial performance traits.

Furthermore, *A. delalandii* outperformed *X. laevis* in both speed and acceleration traits in both burst swimming and hopping experiments, despite *X. laevis* being adapted to an aquatic lifestyle.

Lastly, I implemented ecophysiology models using the results of the temperature-trait relationships obtained in chapter four and five by constructing spatially explicit surface models which were used as input layers in *Maxent*. Following the same methods as in chapter three, I found that ecophysiology modelling techniques accurately predict current distributions of these widely distributed African anurans. Although species have lost suitable climate space in the past, models predict that *A. delalandii* will gain thermally suitable space by the year 2080 while *X. laevis* is also expected to gain suitable thermal space only under MPI-ESM GCM. *Sclerophrys capensis* is expected to lose suitable climate space in the same period under all GCMs.

In conclusion, I showed that although correlative-based SDMs are useful, bottom-up trait-based techniques such as ecophysiology models improves our understanding on how large-scale climate variables affect key physiological traits in shaping what we observe at species distribution level. In addition to biological traits and ecological specialisations, the incorporation of climate variation in modelling processes is also a necessary aspect, given that we have no clear understanding of how climate change will eventually take place.

Opsomming

Een van die grootste bedreigings vir anuran diversiteit teen die einde van die eeu is Klimaatsverandering. Wat anurans anders as ander gewerweldes maak, is hul kenmerkend klein verspreidingsgebiede en beperkte verspreidingsvermoëns. Om te voorspel hoe klimaatsverandering anurans gaan beinvloed, het wetenskaplikes die korrelatiewe-gebaseerde spesieverspreidings modelle (SDMs) gebruik. Tog word hierdie metode gekritiseer vir die simplistiese benadering van korrelasie van bekende verspreidings van klimaat veranderlikes.

Die doel van hierdie studie is om met behulp van 'n multi-dissiplinêre benadering, inligting uit verskeie areas (fisiologie, prestasie en morfologie) te inkorporeer in SDMs om te help met ‘n meer akkurate voorspelling vir Suider-Afrikaanse amfibiese diere met verskillende lewens-kenmerke vir klimaatsverandering. Eerstens het ek die SDM metode gebruik om die impak van klimaatsverandering op die verspreidingsgebied van 37 nouverspreide spesies anuran van die Kaap Floristiese Streek vas te stel. Ek het korrelatiewe-SDMs gebruik vir huidige kondisies en dit gebruik op 2 tydperke in die verlede naamlik i) die Laaste Ystydperk Maksimum (LYM) en ii) die Holoseen Ystydperk Minimum (HYM). Ek het ook die metode gebruik om die tydperk tot 2080. My bevinding was dat die biotiese snelheid waarteen die KFS amfibiese gemeenskap na verwagting sal skuif is veel vinniger as historiese verpsreidingsgroei. Hierdie modelle dui verder daarop dat die KFS amfibiese gemeenskap reeds ≈ 56% verloor het van geskikte klimaatruimte sedert die LYT en dit sal na verwagting versnel onder toekomstige scenarios.

Proef drie: Met soortgelyke metodes as hierbo, word die impak van klimaatsverandering vasgestel op die verspreidingsgebiede van drie wyd verspreide anuran spesies met verskillende ekologiese agtergronde: *Xenopus laevis*, *Amietia delalandii*, en *Sclerophrys capensis*. Ek het korrelatiewe gebaseerde SDM modelle gebruik vir huidige toestande en vir die LYM en om 2080 te voorspel. Alhoewel al drie spesies geskikte klimaat sedert die LYM verloor het, dui daarop dat *A. delalandii* sal na verwagting geskikte klimaat ruimte te bykry, ten minste onder twee ASM naamlik CCSM (25%) en MPI-ESM (7%), terwyl *S. capensis* en *X. laevis* verwag om geskikte klimaatruimte te verloor tot 2080.

Proef vier het ek fisiologiese eksperimente gedoen om die rol van omgewingstemperatuur (T_a) en liggaamsmassa (M_b) op liggaamstemperatuur (T_b), standaard metaboliese tariewe (SMR) en verdamping waterverlies (EWL) te bepaal. Liggaamstemperatuur verhoog met 'n toename in M_b in *A. delalandii* en *S. capensis* maar nie in *X. laevis*. Ek het egter nie 'n beduidende effek gevind van beide M_b en seks op die geheel-dier EWL in al drie spesies. Geheel-dier SMR verhoog met 'n toename in M_b in net *S. capensis*.

Proef vyf dan bepaal ek hoe veranderinge in temperatuur die volgende beïnvloed: i) spring - swem (snelheid en versnelling) en uithouvermoë (afstand en tyd) in verskillende. Ek het gevind daar was beduidende verskille in spesies reaksie norme net in terrestriële prestasie eienskappe. *A. delalandii* het beter gevaar as *X. laevis* in beide die spoed en versnelling eienskappe in swem en spring eksperimente, ten spyte daarvan dat *X. laevis* aangepas is vir akwatiese leefwyse. *Sclerophrys capensis* het beter gevaar as *X. laevis* en *A. delalandii* in spring uithouvermoë proe (afstand en tyd), ten spyte van veranderinge in T_a.

Laastens, het ek die resultate gebruik van hierdie temperatuur-eienskap (dit wil sê, fisiologie, prestasie en morfologie) verkry in proef drie en vier in 'n ekofisiologie modellering platform. Hierdie temperatuur-eienskap verhoudings is gebruik om ruimtelik eksplisiete oppervlak modelle te bou wat gebruik word om inset lae tot huidige spesie verspreiding met behulp van *Maxent* model te bou. Ek het toe hierdie modelle gebruik op verspredings soortgelyk aan ASM as in proef drie. Die resultaat was dat die ekofisiologie modelleringstegnieke die verspreiding van wyd-verspreide Suid-Afrikaanse anurans akkuraat kan voorspel. Van belang is dat die modelle dui daarop dat *A. delalandii* meer klimaatgeskikteruimte sal verkry teen die jaar 2080, met al ASM ingesluit, terwyl *X. laevis* ook na verwagting meer geskikteklimaatruimte sal verkry, maar net onder MPI-ESM ASM. *Sclerophrys capensis* sal na verwagting geskikteklimaat ruimte verloor in dieselfde tydperk onder al die ASM.

Ten slotte, Ek het getoon dat, hoewel korrelatiewe SDM's korrek is, onderliggende eienskappe soos ekofisiologie modelle ons begrip verbeter oor hoe grootskaalse klimaatveranderlikes belangrike fisiologiese eienskappe beïnvloed in die vorming van wat ons waarneem op spesieverspreidingsvlak. Benewens biologiese eienskappe en ekologiese spesialisasies, is die inkorporering van klimaatsvariasie in modelleringsprosesse ook 'n noodsaaklike aspek, aangesien ons nie verstaan het hoe klimaatsverandering uiteindelik sal plaasvind nie.

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Chapter one: Amphibians in a changing climate

Introduction

Changes in the global climate are not a new phenomenon and are known to have influenced species persistence and extinctions rates throughout the history of the earth (Hewitt 2000; Petit et al. 2008; Wake & Vredenburg 2008). Although the world has experienced climatic changes in the past, resulting from Milankovitch cycles (Compton 2011; Zachos et al. 2001), these have followed series of glacial and interglacial periods (Hays et al. 1976). In keeping with these climatic cycles, there are clear indications that the earth's climate should be in a cooling trend since the last glacial minimum (Holocene glacial minimum: HGM) which occurred about 6 000 year ago. This was indeed the observed trend with the little ice-age around the 12th century but this cooling trend occurred until the 1930's, when there was a sudden increase in mean global temperatures (e.g., Mann et al. 1999; Mann et al. 2008) and it has been on a warming trend ever since, at least for the northern hemisphere (Mann & Jones 2003). Moreover, the last 40 years that have been characterised by very steep increases in temperature, have also been reported as the warmest years in the last millennium (at least for the Northern Hemisphere; see Mann et al. 1999). Models for the southern hemisphere on the other hand have been less conclusive owing to the scarcity of reconstructive climate proxy data studies (e.g., sediment core extracted from lake, deep sea or ice) in the region (Jones et al. 1999).

Studies on past climate change events suggest that changes have occurred at varying rates (Ammann et al. 2000; Overpeck & Cole 2006; Williams et al. 2002) but these have allowed species to respond to changes (Willis et al. 2013). One of the primary ways in which species have responded to climatic changes has been through altering their distribution ranges and by moving to climatically suitable areas (Davis & Shaw 2001; Dynesius & Jansson 2000; Parmesan & Yohe 2003). Theoretically, contemporary species could also move to climatically suitable areas but the extent to which landscapes are currently transformed will prove detrimental to facilitating such movements (Hannah et al. 2002; Midgley et al. 2006). What is particularly noteworthy about the recent anthropogenically-induced climate change is that two of the earth's systems, the carbon dioxide (Hoerling et al. 2006; Overpeck & Cole 2006) and the ocean's pH (Mora et al. 2013), have moved out of their "normal" envelope that has existed for the past 2 million years. Surface temperatures also are expected to increase continuously in a warming trend by 2069 (Mora et al. 2013).

It is predicted that the current changes in climate, will far exceed historical rates, particularly at the regional scale. Future predictions for southern Africa suggest that increases in the drying pattern of the region, which started in the 1950's, will continue well into this century (Giannini et al. 2008; Hoerling et al. 2006). Climatic forecasts for South Africa suggests a drying trend in the current winter rainfall area, resulting from the increased drying of austral winter. This is caused by the increase in both Indian and Atlantic sea surface temperatures and the El Niño Southern Oscillation (ENSO), two of the most important systems affecting precipitation levels in southern Africa (Adler et al. 2008; Hoerling et al.

2006). Warmer conditions coupled with increased precipitation, a pronounced increase in spring temperatures as well as an increase in the number of dry days for both spring and summer seasons is predicted for the eastern parts of southern Africa (Davis 2010; Engelbrecht et al. 2013). Furthermore, temperature increases in the interior are expected to be more pronounced than those predicted for coastal areas, with expected increases of between 1-3° C towards the end of this century and a further increase between 3-5° C by the next century (Davis 2010), although some models also predict increased rainfall for the interior of the region (Engelbrecht et al. 2011).

Amphibians make for an interesting vertebrate group to assess the impacts of climate change on species distributions because first, their distribution in sub-Saharan Africa is primarily driven by differences in environmental temperatures, while the variation in rainfall patterns plays a secondary role (Poynton 1964). Second, they are the most threatened (Pimm et al. 2014) and least conserved vertebrate group owing to a high proportion of narrowly distributed endemics that characterise this group (Rodrigues et al. 2004). Third, most amphibians have poor dispersal abilities (Wollenberg et al. 2011; Zimkus et al. 2012). In cases where species have large distribution ranges, populations within this range are highly structured, such that the proportion of gene exchange between populations is low (Furman et al. 2015; Tolley et al. 2010), an important factor if species are to adapt to changes in climate (e.g., Davis and Shaw 2001). Lastly, most amphibians display a bi-phasic life style as well as a high variation in life-history traits. This variation in life-history traits leads to a variation in ways each group will be affected by threats (Becker et al. 2007; Becker et al. 2018; Mokhatla et al. 2012; Morrison & Hero 2003; Whitfield et al. 2007).

To assess the predicted impacts of climate change on vertebrate ectotherms, correlation-based Species Distribution Models (SDMs) have been conducted for Europe (Araújo et al. 2006) and south-east Asia (Bickford et al. 2010) and sub-Saharan Africa (Garcia et al. 2014). These studies concluded that vertebrate ectotherms (particularly amphibians), are expected to i) lose suitable climate space because the rate of change will be far more rapid than these taxa can adapt, and ii) these taxa will face species extinctions as climate change acts in concert with habitat transformations (Hof et al. 2011). The underlying assumption under future climate conditions, is that amphibians will be worst affected, as reptiles are not limited by the availability of water and as thus, have evolved to inhabit drier habitats, made possible by the evolution of the amniotic eggs and scales (see Araujo et al. 2006).

Most of the work conducted on southern African species has focused on plants in the Cape Floristic Region (CFR: Hannah et al. 2007; Hannah et al. 2005; Midgley et al. 2006; Rutherford et al. 1999), a multi-taxon treatment by Erasmus et al. (2002), endemic birds (Coetzee et al. 2009) and reptiles (Houniet et al. 2009). Consensus from these correlation-based species SDMs is that the South African fauna is expected to shift eastward, while the flora, particularly of the south-western Cape is expected to shift both east and southward (Erasmus et al. 2002; Rutherford et al. 1999). Moreover, the common

attribute from all these species range shifts is that most of these taxa are expected to lose climatically suitable areas, which will also lead to an increase in the spatial mismatch with the current conservation portfolio (Hannah et al. 2007).

Work on the South African anuran fauna is now underway to help us understand the role of climate (both previous and current) on species and their responses. (Schreiner et al. 2013) assessed how previous climate regimes might have acted in shaping the distribution of frog species in the CFR and Kwa-Zulu Natal. In a recent study, Botts et al. (2012) found that over 60% of southern African frogs have experienced distribution range reductions and that climate specialists had proportionately small distribution ranges compared to environmental specialist (Botts et al. 2013). Moreover, Hof et al. (2011) suggested that global climate changes as well as human landscape modifications are likely to be more detrimental to the survival of amphibians (at least going forward) particularly in southern Africa. South Africa has good distribution (spatially determined) anuran data, through the frog atlas project completed in 2004 (SAFAP: Minter et al. 2004). In addition to this spatial dataset, southern Africa has a very unique and rich amphibian fauna, with 12 out of 15 sub-Saharan anuran families represented in South Africa. Furthermore, the amphibian fauna of the south-western Cape has recently been identified as one of the important faunal assemblages due to its markedly high levels of phylogenetic turn-over of frogs at a global level (Holt et al. 2013). The high level of species diversity means that amphibians also display a number of different life-history traits, habitat specializations, physiology and behaviour (Hillman et al. 2009).

Conceptual framework

It is evident that changes in the global climate have had a profound impact on the distribution of species in the past (Ammann et al. 2000; Henrici & Báez 2001), currently (Pounds et al. 2006; Pounds et al. 1999) and they will continue to shape species distributions going into the future (Araujo et al. 2006; Bickford et al. 2010). Understanding patterns of current species distributions (biogeography) and factors driving these patterns will help us in accurately predicting these changes in the future and devising appropriate conservation actions. Indeed, this has formed the basis of correlation-based SDMs. However, the application of these models has been limited mainly by the assumption that all species are similar in their life-histories and thus they will have a single response to any climate change scenario (see Bellard et al. 2012; Thuiller et al. 2006). To address this, advances in SDMs have moved towards incorporating physiological, morphological, behavioural and performance data in accurately determining current distribution range of species and how are these expected to change in future (see Figure 1.1).

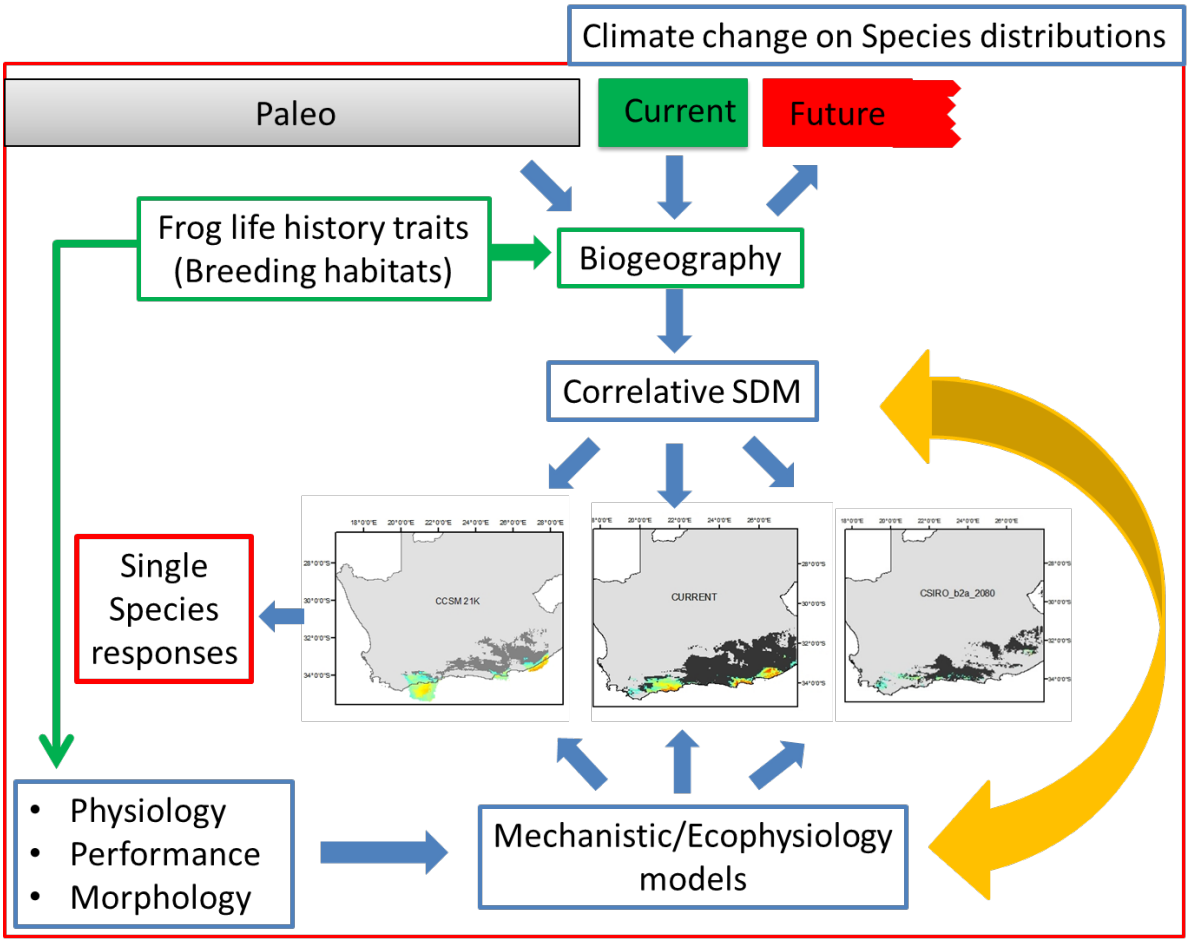

Figure 1.1. A representation of the conceptual framework adopted in this thesis. The blue boxes represent different chapter making up this thesis and the arrows show how different chapters link with one another. Green boxes represent input factors and the red boxes represent drowbacks and uncettain outcomes from the modelling process as a result of an uncertain future, complexity of the modelling process and the inherent modelling shortfalls.

Aims and objectives

Recent advances in SDMs (Kearney & Porter 2004; Sinervo et al. 2010) and assessing the invasive potential of non-native species (Kearney et al. 2008; Kolbe et al. 2010) have incorporated physiology, performance, as well as behaviour into modelling processes through ecophysiology (e.g., Sinervo et al. 2010) as well as mechanistic (e.g., Kearney and Porter 2004) modelling techniques. These studies highlighted that different life stages have different physiological limitations (see Kearney et al. 2008; Kolbe et al. 2010), thus suggesting climatic changes will affect each life stage differently. But to my knowledge, no study has looked specifically at how anuran species with different ecologies will be affected by climate change (Bellard et al. 2012; Thuiller et al. 2006; see also Foden et al. 2013; Garcia et al. 2014 although these studies focused on broad scale variables ideal for analysis at a global scale). The aim of this study is to synergise data from multiple lines of evidence (morphology, physiology and performance) and incorporate them into SDMs to aid in predicting the responses of different, widely

distributed, largely temperate African anuran species to climate change. Such data have rarely been merged into SDMs; despite all contributing to the range, dispersal and survival of target organisms (Bellard et al. 2012; Thuiller et al. 2006). The main objective is to produce a suite of ecophysiology models for different anuran species with different ecologies to make more reliable predictions on the shifts of biological communities in response to climate change. Due to the wide range of body forms, life-histories and even a wider variation in habitat specialisation, it is unlikely that all anurans would have a single response to any given climatic scenario. Using correlative-based SDMs, the first aim is to determine the impact of climate change on the past, current and future distribution ranges of narrow-range endemic anuran species of the Cape Floristic Region. The second aim is to determine the past, current and future climate change on widely distributed, temperate anuran species, namely: i) the largely aquatic African clawed frog (*Xenopus laevis*), ii) semi-aquatic, common river frog (*Amietia delalandii*) and iii) the largely terrestrial raucous toad (*Sclerophrys capensis*). Thirdly, I want to assess how changes in temperature affect the physiology (standard metabolic rated and evaporative water loss) of the three-temperate species. Fourthly, the aim is to determine if the variation in temperature affects how species outperform each other as a function of species'ecology. Lastly, to incorporate physiology, performance as well as morphology into ecophysiology models, to better predict the impact of climate change on the distribution of widely distributed, temperate African anuran species with different ecologies.

Chapter two: Assessing the effects of climate change on distributions of Cape Floristic Region amphibians †

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Abstract

Climatic changes have had profound impacts on species distributions throughout time. In response, species have shifted ranges, adapted genetically, behaviourally and/or become extinct. Using Species Distribution Models (SDMs), we examined how changes in suitable climatic space could affect distributions of 37 endemic frog species in the Cape Floristic Region (CFR), an area proposed to have evolved its megadiversity under a stable climate, but expected to change substantially in future. Species distributions were projected onto mean climate for current (1950 to 2000), hind-casted to paleo-climate (Last Glacial Maximum; LGM ≈ 21 Kya and Holocene Glacial Minimum; HGM ≈ 6 Kya) and forecasted for two emissions scenarios (A2a and B2a) for the year 2080. We then determined the changes in area sizes, direction (longitude and latitude), fragmentation index, biotic velocity and assessed if these were affected by life-history traits and altitude. We found that the biotic velocity at which the CFR amphibian community is expected to shift North (A2a ≈ 540.5 km/Kya) and East (B2a ≈ 198 km/Kya) far exceeds historical background rates (≈1.05 km/Kya, North and West ≈ 2.36 km/Kya since the LGM). Our models further suggest that the CFR amphibian community has already lost ≈ 56% of suitable climate space since the LGM and this is expected to accelerate under future emission scenarios (A2a ≈ 70%; B2a ≈ 60%). Lastly, we found that highland species were more fragmented than lowland species between the LGM and current, but the fragmentation of lowland species between current and future climates is expected to increase.

Introduction

Climatic changes have had profound impacts on the survival and extinction rates of species through time (Wake & Vredenburg 2008). One of the ways in which species have dealt with these climatic changes has been through adjusting their distribution ranges (Dynesius & Jansson 2000), although changes in behaviour and genetic adaptation can also play a role (Davis & Shaw 2001; Jump & Penuelas 2005). Evidence from ice cores and other climate reconstruction studies (Compton 2011; Zachos et al. 2001) have provided better insight into how past climatic changes may have structured distributions as climates were changing. Recent evidence from fossil records suggests that past climatic fluctuations may have brought about changes at broad scale regional/biome level. For example, the occurrence of lakes and "forest-elements" within the current Sahara region (between 11 and 4 Kya, under warmer and wetter conditions) and the replacement of forest by savannah vegetation in equatorial west Africa when conditions are predicted to have been warmer and drier than present (Willis et al. 2013).

Studies undertaken in southern Africa, particularly in the Cape Floristic Region (CFR) suggest that climate has remained relatively stable throughout the Quaternary (Compton 2011; Cowling et al. 1996). Indeed, areas such as the CFR that are proposed to have experienced relatively stable climate coincide with contemporary centres of richness, and narrowly distributed endemic species (Dynesius & Jansson 2000). This is because these areas may have served as climate refugia during times of climatic perturbations (Araújo et al. 2008; Jansson 2003). Moreover, such areas are also proposed to have experienced relatively slow climate change velocities (Loarie et al. 2009; Sandel et al. 2011). Conversely, areas that have experienced rapid changes in climate are suggested to harbour biota that have coupled biotic velocities: the velocity of shifts in species' climate envelopes (Ordonez & Williams 2013). Understanding how communities respond to these changes is very important given the biological importance of megadiverse regions under threat (Myers et al. 2000). Amphibians of the CFR in particular provide an excellent group to study the role of climate in structuring species community distribution because: i) they are biogeographically distinct at a global scale (Holt et al. 2013) and ii) amphibians are generally considered as poor dispersers (Tolley et al. 2010), and are thus more directly affected by changes in climate compared to other vertebrates.

While attempting to elucidate the importance of ecological factors in describing amphibian species distribution patterns in southern Africa, Poynton (1964) hypothesised that the climatic oscillations resulting from Milankovitch cycles would have driven the expansion of the CFR amphibian assemblage inland as a response to an increase in climatic suitability during glacial periods. Dynesius & Jansson (2000) suggest that large scale climatic oscillations may have favoured species with large distribution ranges, because these species were able to migrate to climatically suitable areas or persist in climatically unsuitable areas (see also Van Bocxlaer et al. 2010). Furthermore, other past climate models for the CFR argue that during glacial periods, the extent of the CFR's winter-rainfall area increased as a result

of westerlies migrating north towards the equator, providing moisture to areas of the current Karoo region (Chase & Meadows 2007), thus supporting Poynton's hypothesis. Until recently, it had been very difficult to test such hypotheses. However, using Species Distribution Modelling (SDM) techniques, a recent test of Poynton's hypothesis concluded that he was correct in predicting range expansion of the CFR assemblage during glacial periods (Schreiner et al. 2013). Nevertheless, instead of expanding inland, the assemblage was predicted to have made use of the exposed Agulhas bank, during periods when sea level was much lower (Compton 2011).

Future climate models for southern Africa predict increased drying in the winter rainfall region (Engelbrecht et al. 2013; Giannini et al. 2008), caused by the increase in both Indian and Atlantic Sea Surface Temperatures and the El Niño Southern Oscillation (Hoerling et al. 2006). Understanding how changes in climate may have structured distribution is of particular relevance as it will help in: i) predicting how current changes in climate might affect distributions as well as interactions among species (Blois et al. 2013) in the CFR and ii) adequately planning for these changes considering the climatic history of the region (Dynesius & Jansson 2000) and other biodiversity threats such as habitat modification (Newbold et al. 2015).

The aim of this study is to determine how climate (paleo-, current and future) affects the distribution of the CFR endemic amphibian community. To do this we take an ensemble modelling approach using General Circulation Models (GCMs) of two paleo-climates and four future climate GCMs, projected under two emission scenarios. In particular, we determine how the distributions of the CFR amphibian community have changed in terms of: i) direction (latitude and longitude), ii) area of occupancy (changes in areas occupied at different climate periods), iii) biotic velocities (the rate at which communities, at a regional level shift with changes in climate, see Ordonez & Williams 2013) and iv) fragmentation. Furthermore, we estimate how life-history traits and altitudinal specialisation affect these outcomes. We assume that shifts (direction of movement and size) in climatically suitable space across climatic periods will lead to coupled species range shifts in CFR amphibian community (Schreiner et al. 2013).

Methods

Data

The most recent taxonomic treatment of species was used to ensure that all species of interest were included (Channing & Wahlberg 2011; Frost et al. 2006; Turner & Channing 2008; Turner et al. 2004). Approximately 40 species are endemic to the Cape Floristic Region (CFR; here taken as areas representing both the Fynbos and the Succulent Karoo biomes; Figure 1C: Colville et al. 2014). We excluded three recently described species: i) *Breviceps branchi* (Channing 2012), ii) *Cacosternum*

aggestum and iii) *C. australis* (Channing et al. 2013) because of the relative lack of distribution records, leaving 37 CFR endemic amphibian species from 6 families for further analysis. As some amphibians do not display a bi-phasic typical amphibian life style, for example, members of the direct-developing genus *Arthroleptella* endemic to the CFR (Minter et al. 2004), this leads to a variation in ways each life-history trait will be affected by threats (Mokhatla et al. 2012). To assess the effect of life-history traits on species distributions resulting from changes in climate, species were grouped according to their modes of egg deposition and development (Wells 2007: Supplementary Information Table S2.1).

There was a high variation in the number of available species distribution records, from *Cacosternum platys*, Rose 1950 (4 records) to *Tomopterna delelalandii*, Tschudi 1838 (541 records). Occurrence records were obtained from the South African Frog Atlas Project (SAFAP: Minter et al. 2004), CapeNature, I-Spot as well as records published in recent literature (Channing & Wahlberg 2011; Measey & Tolley 2011; Turner et al. 2004) and our own observations. We only included records with accuracy to the nearest second or higher. Records were projected into Arc Map GIS 10.2 (ESRI 2014) to identify any doubtful records that may be recorded outside known ranges, using Global Amphibian Assessment (GAA 2008, IUCN; http://www.iucnredlist.org/ accessed September 2013) polygons as 'know distribution ranges'. Based on these, we judged whether the outlying records represented credible sightings or should be excluded from further analyses.

Climate data

Environmental predictors were compiled from a set of 19 bioclimatic variables at a spatial resolution of 2.5 arc minutes available from WorldClim (Hijmans et al. 2005). Inter-correlation structure among predictors throughout the study area was assessed by computing pairwise squared Spearman's rank correlation coefficients, and in those cases where R^2 exceeded 0.75, only the putatively biologically most important variables were retained. Ten variables were retained after computing the Spearman's rank correlations: isothermality (mean diurnal range/temperature annual range; BIO3), temperature annual range (BIO7), temperature of wettest quarter (BIO8), mean temperature of driest quarter (BIO9), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), precipitation of wettest month (BIO13), precipitation seasonality (BIO15), precipitation of driest quarter (BIO17) and precipitation of coldest quarter (BIO19); see Supplementary Information (Table S2.2) for percentage variable contributions.

Species Distribution Modelling

Species distribution model projections were created at a 2.5 arc minute resolution, using current distribution data to reconstruct hypothetical distributions for paleo- and future climate. To reconstruct past distributions, we projected SDMs onto paleo-climate for the Last Glacial Maximum (LGM ≈ 21 Kya) and the Mid-Holocene/Holocene Glacial Minimum (HGM ≈ 6 Kya) climate periods. We used 2 GCMs: the Community Climate System Model (CCSM: Otto-Bliesner et al. 2006) and the Model for Interdisciplinary Research on Climate (MIROC: Hasumi & Emori 2004), available through http://pmip2.lsce.ipsl.fr/ (Braconnot et al. 2007). Paleo-climate data were downscaled to a spatial resolution of 2.5 arc minutes following the delta method (Peterson & Nyari 2008). Current distributions were based on SDM projections onto mean climate from 1950 to 2000. To forecast future distributions for the year 2080, SDMs were projected using 4 GCMs: i) General Circulation Global Climate Model (CGCM2: Flato & Boer 2001), ii) Hadley Centre Coupled Model (HadCM3: Gordon et al. 2000), iii) Commonwealth Scientific and Industrial Research Organisation Mark-2b (CSIRO-Mk2: Gordon & O'Farrell 1997) and iv) National Institute for Environmental Studies (NIES 99: Emori et al. 1999); to capture the variability associated with predicting climate change. Each GCM was projected onto two Intergovernmental Panel on Climate Change emission scenarios, both representing a more heterogeneous world: i) A2a representing a business as usual climate outlook with average temperature increases between 2° and 5.4° C and ii) B2a representing a more optimistic outlook with moderate increase in future temperature between 1.4° and 3.8° C (Nakicenovic & Swart 2000).

The selected environmental variables were used to model both past and future potential distributions of amphibian species using an ensemble species distribution modelling framework, BIOMOD2 (Thuiller et al. 2013), a multi-model platform implemented in R (R Development Core Team 2017). The BIOMOD2 procedure uses an ensemble of modelling techniques including Generalized Linear Models (GLM), General Additive Models, Generalized Boosted Models, Classification Tree Analysis, Artificial Neural Networks, Surface Range Envelope, Flexible Discriminant Analysis, Multi Adaptive Regression Splines, Random Forests, including Maximum Entropy to produce consensus models, which are weighted averages between all models (Marmion et al. 2009). To ensure that our models were comparable across scale, they were rescaled using a binomial GLM.

We randomly selected 1000 pseudo absence records, three times within a circular buffer of 100 km enclosing the species records as environmental background. For model evaluation, we divided the species records into 80% used to train the models and 20% for model evaluation using three different performance criteria: i) area under the receiver operating characteristic curve: AUC Swets 1988), ii) true skills statistics and iii) Cohen's Kappa (Allouche et al. 2006). As a quality threshold for the final ensemble, we selected only those models with an AUC score >0.7 (see Supplementary Table S2.3). The final ensemble was built using a proportional weighting of the models according to their predictive performance. As a presence-absence threshold, we selected the minimum training presence as non-fixed threshold (Liu et al. 2005). When projecting the ensemble across space and time, non-analogous

climatic conditions that exceeded the training range of the models were quantified wherein the numbers of these predictors are highlighted using Multivariate Environmental Similarity Surfaces (MESS: Elith et al. 2011; see Supplementary Figure S1.1 paleo-, current & Supplementary figure S 1.2: future projections for *Arthroleptella bicolor* as an example). Projections into non-analogous climates may increase the potential for extrapolation and interpolation errors (Elith et al. 2011). Potential areas of occurrence were computed based on presence-absence maps applying the minimum training presence threshold using the respective functions in the raster package for R (i.e., 'area') but excluding extrapolation areas as identified by MESS. To determine the final consensus area projection for each climate period across all models (different GCMs), we projected all models on to Arc Map GIS 10.2 (ESRI 2014) and the consensus was taken as a combination of all grid cells that were identified as suitable by 50% or more of the models at each climate period (Forester et al. 2013).

Statistical analyses

To assess changes in species distributions across climatic periods, we calculated the total area, perimeter and the centroid coordinates for each species across climatic periods from suitable climate space projections. Area and Perimeter were then used to determine the fragmentation index (i.e., Landscape Dissection Index, DI´: Bowen & Burgess 1981). This was achieved by determining the edge of each species distribution range, in relation to the core of the distribution using the formula:

$$DI' = \sum_{i=1}^{n} P/(2\sqrt{\pi \sum_{i=1}^{n} A})$$

Where DI´ is Landscape Dissection Index, *P* is Perimeter and *A* is the Area; DI´ compares the amount by which any "island" or a distribution range is dissected relative to the total area, with a DI´ of 1 representing a circle, suggesting a less fragmented shape with a low Perimeter to Area ratio. We determined changes in area occupancy, rate of area change, the direction of range shifts (across both longitudes and latitudes) and fragmentation index (DI´) between climate periods (VanDerWal et al. 2013; Wollenberg et al. 2011). We ran our results in two ways, first with a full suite of 37 species and secondly with 34 species (excluding species with less than 10 species records to avoid model over-fitting, (see Platts et al. 2014). We found that the results in both attempts were almost identical and that patterns remained largely similar and thus report results of the analysis considering a full suite of 37 species. Lastly, we checked whether these changes are affected by altitude and life-history trait. All the tests were performed in R (R Development Core Team 2017).

Results

Range shifts

Our results suggest that the amphibian community of the CFR would have responded to Quaternary climatic changes by shifting distributions. Longitudinally, we found that between the LGM and current periods, amphibian communities have shifted their distribution northwards, and are expected to shift further northwards, for both A2a and B2a emission scenarios. Biotic velocity has been relatively slow since the LGM to current, but it is expected to increase dramatically if amphibian communities are assumed to track climate suitable space under anthropogenically-induced climate change towards the end of this century (Figure 2.1 A; e.g., LGM – HGM = 1.058 km/Kya compared to Current – A2a = 540.554 km/Kya). Across latitudes, our models suggest that the CFR amphibian community has shifted its range westward between the LGM and HGM, as well as between the HGM and current. However between current and 2080, our models suggest that the CFR amphibian community will shift eastward under both A2a and B2a emission scenarios, although we found this shift between climate periods to be significant for the B2a emission scenario only (Paired-t = 2.119; P = 0.039). Biotic velocity across latitudes between current and B2a climate periods is expected to occur at rapid rates compared to historical rates (see Figure 2.1.A; LGM – HGM 2.360 km/Kya compared to Current – B2a = 198.319 km/Kya).

Changes in area of occupancy

We found that the CFR amphibian community has progressively lost ≈ 56 % of its suitable climate space since the LGM. Furthermore it is expected to lose ≈70 and 60 % of climatically suitable space under A2a and B2a emission scenarios, respectively (Figure 2.2 A). However, we found that the rate at which the CFR endemic amphibian community has lost climatically suitable space has been relatively slow between LGM, HGM and current, but this is expected to increase significantly under future climate change (in particularly, under the A2a emission scenario: Figure 2.1 B).

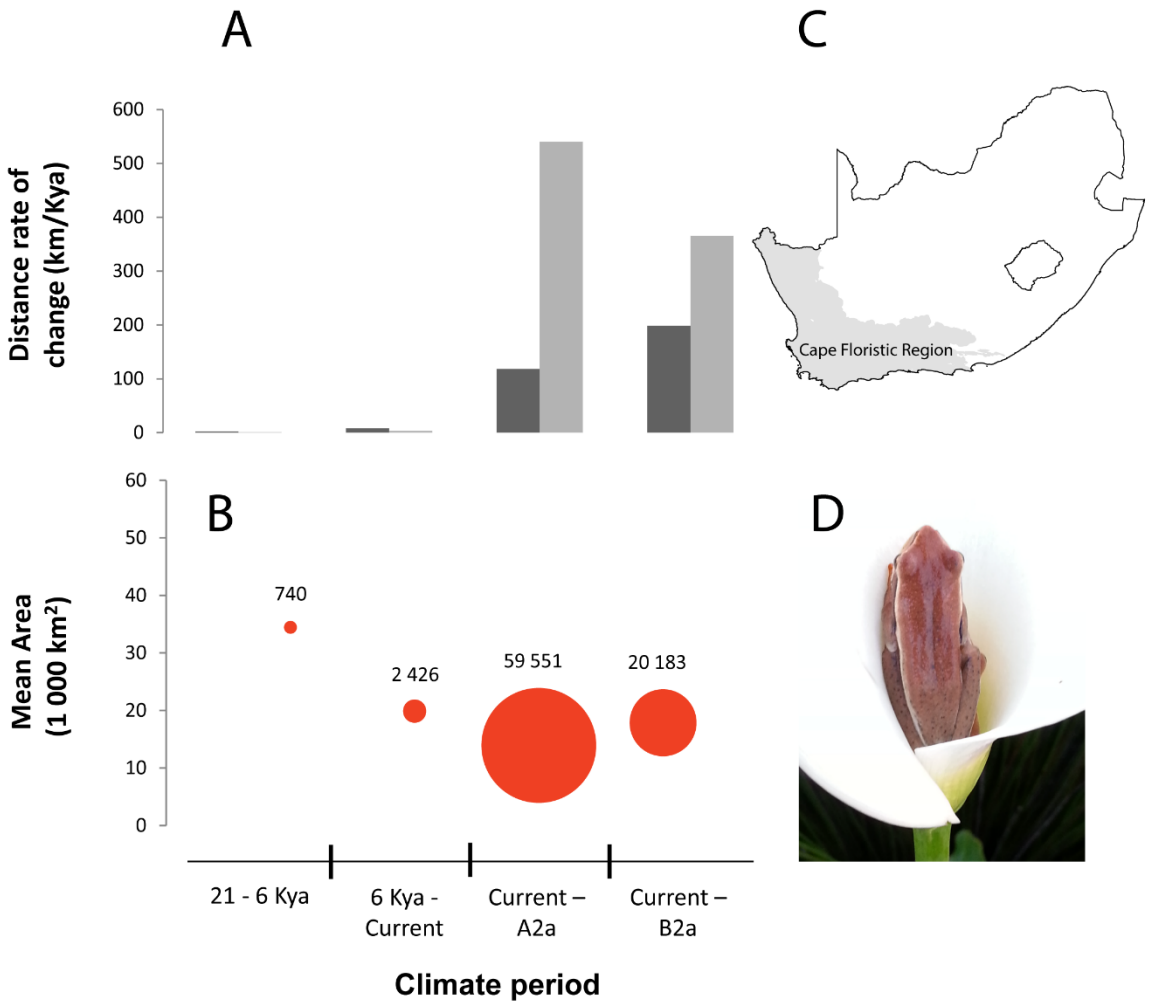

Figure 2.1. Changes in distribution predicted by Species Distribution Models for the endemic amphibian community of the Cape Floristic Region (CFR) *A*: Average Longitude (grey bars) and Latitude (black bars) rate of change between climate periods. *B*: Mean area distributions range size across climatic periods. i) the size of the circle is proportional to the rate of area change within the climate period, ii) the numbers above the circles are the mean predicted rates of area changed between climatic periods in km^2/Kya. *C*: Map of South Africa showing the extent of the CFR (Fynbos and the Succulent Karoo biomes, see methods). *D*: *Hyperolius horstockii*, the Arum lily frog is endemic to the CFR.

Landscape Dissection Index (DI´)

Fragmentation of potential distributions increased slightly from LGM to HGM, but decreased between HGM and current climates (Figure 2.2 B). Our models predict that fragmentation will increase under future scenarios. We did not find any significant difference between life-history traits and DI´. In contrast, we found that the fragmentation of highland species was significantly higher than that of lowland species between HGM and current (Mann-Whitney-Wilcox test; W = 37, *P* = 0.002). However, between current and future climate periods under the A2a emission scenario, fragmentation of lowland species is expected to be higher than that of highland species (W = 18, *P* = 0.033). When looking at

each climate period separately, we found that highland species were significantly more fragmented than lowland species during the LGM (W = 113, *P* = 0.035), HGM (W = 162, *P* = 0.012) and current scenarios (W = 233, *P* = 0.023; see Figure 3). Lastly, we found that the rate at which DI´ changed was only significant between HGM and Current (Paired-t = 2.065; *P* = 0.047).

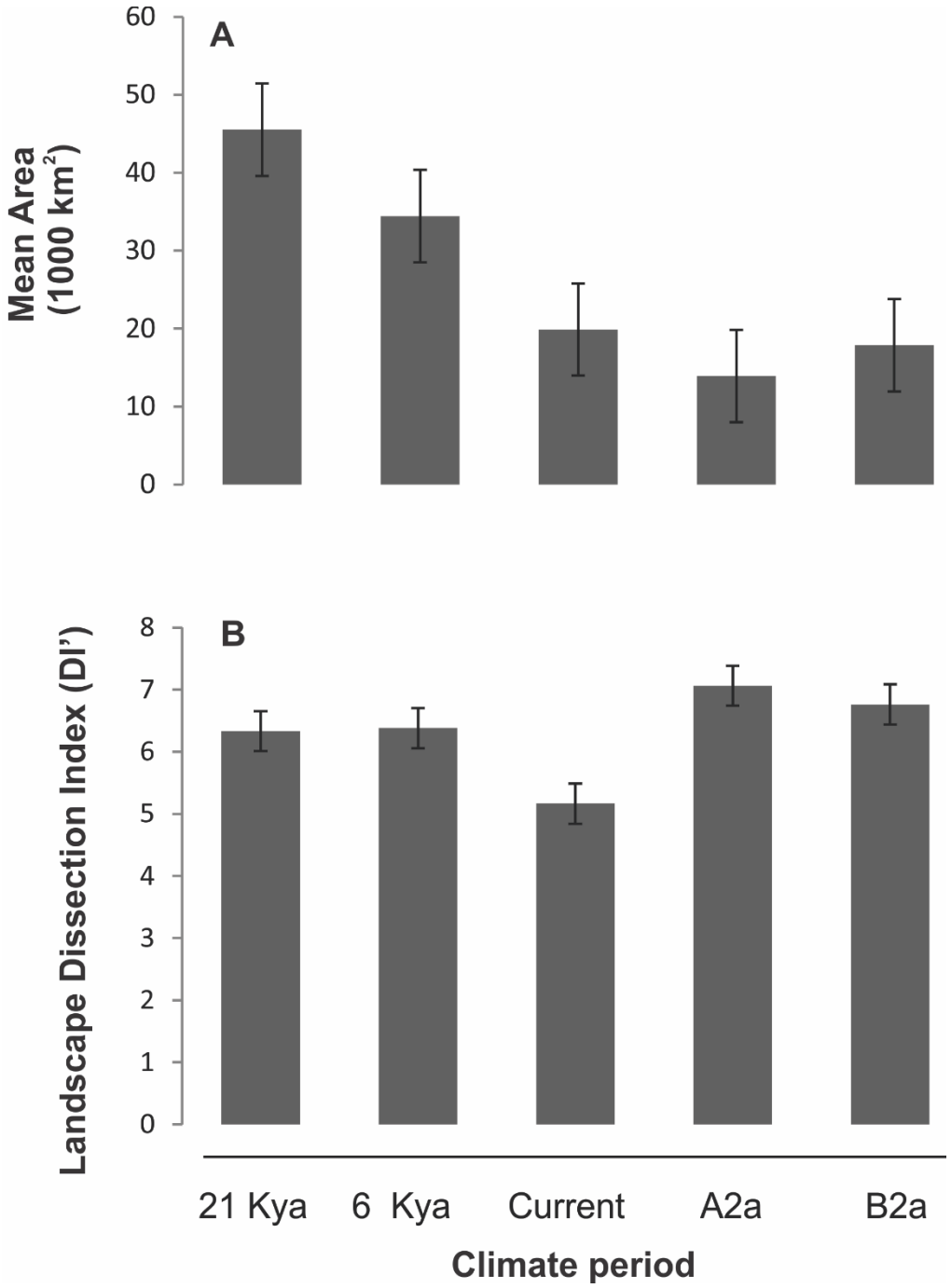

Figure 1.2. *A*: Mean (± standard error) of predicted area occupied by the Cape Floristic Region (CFR) endemic amphibian community suggests that most are already in refugia. *B*: Mean (± standard error) of predicted fragmentation (DI´) in the CFR endemic amphibian community under different climate scenarios.

Discussion

Quaternary climate changes have had significant impacts on the distributions of amphibian communities in the CFR as predicted by Poynton (1964) and modelled by Schreiner et al. (2013). Our results suggest that climatically suitable areas for the CFR amphibian community have shifted northwards since the LGM, and are expected to shift further northwards under both A2a and the B2a future emissions scenarios. Latitudinally, we found that the amphibian community shifted westward from the LGM through to present, but this trend is expected to change to an eastwards directional community shift under both A2a and B2a emission scenarios. Furthermore our models indicate that fragmentation DI´ is expected to increase under future climatic scenarios (Figure 2.2 B) as amphibian communities are predicted to continuously lose suitable climatic space (Figure 2.2 A). These results largely concur with previous studies undertaken in the region (e.g., Erasmus et al. 2002).

One future multi-taxon study which included birds, mammals, reptiles and insects for the whole of South Africa suggested that about 78% of the species included in the analysis are expected to experience range contractions, while 41% are expected to shift their ranges eastward, in response to future climatic changes by the year 2050 (Erasmus et al. 2002). Furthermore, studies assessing the impacts of future climate change in the CFR also predicted an eastward directional shift and concluded that this shift will lead to large scale spatial mismatches with current protected areas portfolio (Hannah et al. 2005). The predicted range shifts and associated impacts are expected to result in as much as 50% loss in current climate space (Altwegg et al. 2014) and as much as 10% of the endemic plant species (Midgley et al. 2002). In addition, we found that the CFR amphibian community has lost ≈ 56% of its suitable climate. Indeed, several studies looking at anurans of southern Africa suggest that they have already lost ≈ 60% of suitable climate space (Botts et al. 2012; Botts et al. 2015), such that current distributions could very well be representative of refugial distributions for most of the endemic Cape species (Schreiner et al. 2013).

We found that fragmentation (as measured by the index DI´) between highland and lowland species was more pronounced during the LGM, HGM and current distributions (Figure 2.3), with highland species being more fragmented than lowland species. When looking at fragmentation between climate periods, we found that the fragmentation index of lowland species is expected to be higher than that of highlands species, suggesting that projected changes in climate will exert pressure on the CFR lowland species in addition to substantial changes in land-use (Bomhard et al. 2005). This result is of significance considering that the CFR lowlands have already been transformed (Bomhard et al. 2005) mainly for agricultural and human settlements, and will become more fragmented in the future as suitable climate space is lost. These results are supported by several studies suggesting that the coupled threat posed by climate change and land-use will be very important, particularly for sub-Saharan African

amphibians in the future (Foden et al. 2013; Hof et al. 2011; Newbold et al. 2015). Furthermore, the suggested increase in fragmentation of lowland species points to the likely increase in movement along altitudinal gradients (Parmesan 2006) as species migrate to highlands in response to projected climate change (Botts et al. 2015). This further emphasises the importance of improving habitat linkages between the CFR highlands and lowlands (Williams et al. 2005), thus retaining the opportunity for altitudinal migrations, which may be critical for poorly dispersing taxa such as amphibians.

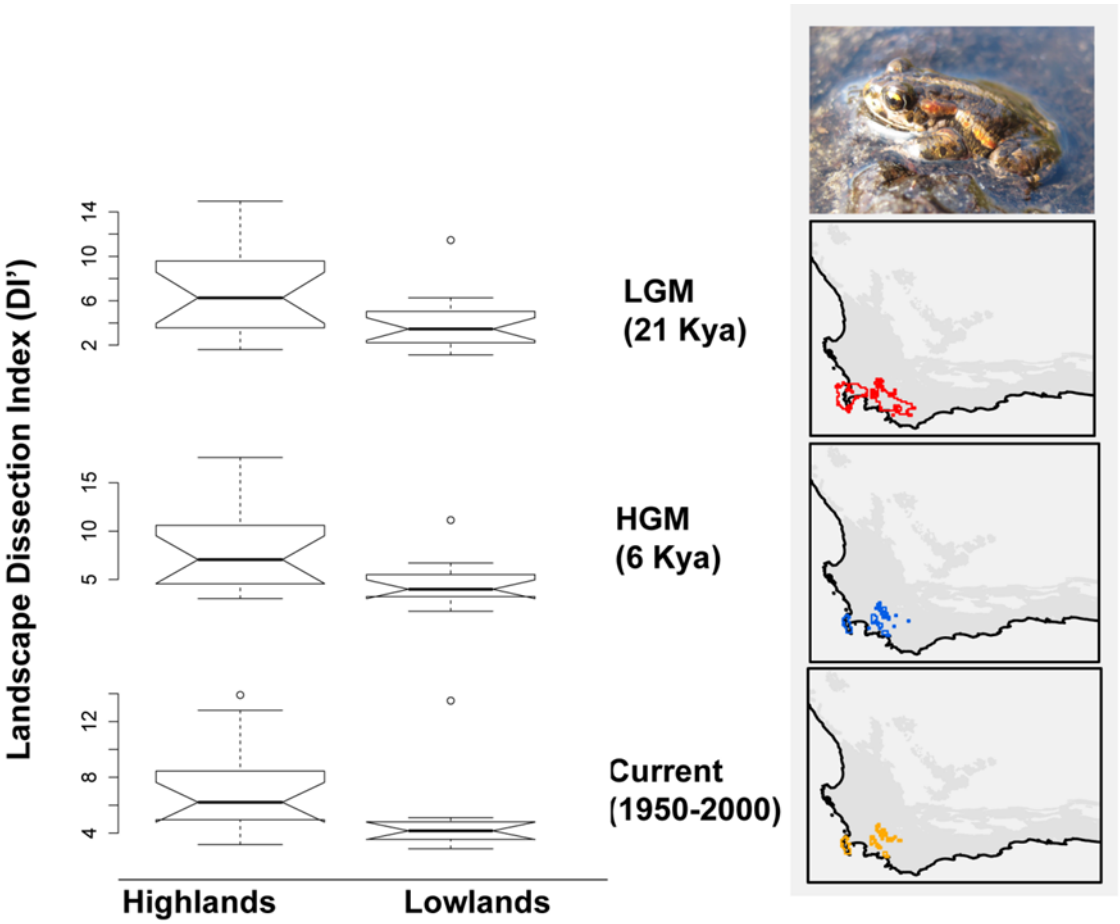

Figure 2.3. Landscape Dissection Index (DI´) compared between Lowland and Highland species of the Cape Floristic Region (CFR) endemic amphibian community during the LGM (red), HGM (blue) and Current (yellow). Hypothetical distribution of a highland species, *Capensibufo rosei* (Rose's mountain toadlet) across climate periods. The figure shows a continuous distribution during the LGM to a more fragmented distribution during the HGM and currently.

Our results, based on future climate change models for the CFR, suggest significant range reductions for the CFR amphibian community as well as rapid rates of suitable area loss. However, certain issues must be taken into consideration when interpreting these results. Firstly, despite their importance in conservation biology, correlative models such as SDM techniques are largely based on the assumption that species distributions are in tune with current climatic factors, and this could lead to an under-estimation of what the real future impact might be (Tolley et al. 2010), specifically when considered in

the light of other biodiversity threats such as habitat destruction (Thomas et al. 2004). Secondly, our landscape dissection index DI´ does not standardise for area size (Thomas et al. 2004). However, controlling for area size in predictive modelling studies may be problematic as these are largely speculative. Nonetheless, these methods are still informative and our predictions are in line with those of previous studies (Botts et al. 2012). Thirdly, the rate of change (as determined in this study) for past and future models, are simple averages taken across climatic periods. We are however of the view that past climatic changes, resulting from Milankovitch cycles, provided species with time to track climatically suitable areas because landscapes were untransformed. We are also aware that species range shifts are but one of several strategies amphibian communities can employ in response to climatic change: other strategies include behaviour, physiology and genetic adaptations (Davis & Shaw 2001; Jump & Penuelas 2005).

In conclusion, we suggest that the results of this study provide important baseline information on a region-wide assessment of the predicted impacts of climate change on a highly diverse vertebrate community of reduced vagility. If we are to reduce the threat of biodiversity loss as a result of climate change within the CFR, the following factors need to be addressed. First, climate change should be included in future protected areas planning efforts (D'Amen & Bombi 2009; Holness & Biggs 2011). Second, efforts aimed at improving the connectivity between highland and lowlands as well as the breeding (e.g., aquatic) and the non-breeding (e.g., terrestrial) habitats of amphibians within the CFR need to be intensified. Third, solutions such as the land stewardship programs entered into with private land owners should be accelerated (Egoh et al. 2010) seeing that most biodiversity is found in private-owned land in the CFR (Gallo et al. 2009). This is particularly important if we consider that amphibians have strong habitat specificity, which is largely driven by their generally poor dispersal abilities (Tolley et al. 2014). This results in deep phylogenetic structures at relatively small spatial scales (Tolley et al. 2010) which could be an outcome of both climate stability and topography (Cowling et al. 2009). We advocate that future models should try to incorporate the use of different GCMs (Forester et al. 2013) because such consensus models out-perform individual GCMs in predicting consensus climatic space. Furthermore, we suggest that, if possible, these should be augmented with mechanistic models (e.g., Kearney & Porter 2009) as species are more likely to use different responses available (behaviour, physiology and range shifts) when dealing with predicted changes in climate

Chapter three: Modelling past, current and future distributions of three temperate African anurans species with different ecological specialisations: a correlative species distribution modelling approach

Introduction

In his seminal work "The biotic divisions of southern Africa as shown by the amphibians", John Poynton remarked on the pivotal role that changes in climate may have played in structuring the distributions of anurans in the region (Poynton 1964). Poynton (1960; 1961) and earlier workers (e.g., Hewitt 1923) highlighted the two major centres of amphibian diversity in the region: the warm, species rich, widely distributed 'tropical' fauna centred on the Mozambique plain, and ii) the cooler, highly endemic, narrowly distributed 'temperate' fauna centred on the southwestern Cape (Poynton 1960). In addition to these prominent centres of anuran diversity (see review by Alexander et al. 2004), Poynton noted the existence of a transitional amphibian fauna and the role that climatic changes might played in structuring its species composition (Poynton 1960). He postulated that during cooler climatic periods such as the glaciation periods, the extent of the temperate fauna would increase further inland along the escarpment and highlands as conditions became favourable (Alexander et al. 2004; Poynton 1962). During warmer inter-glacial climatic periods, he hypothesised that the extent of the tropical fauna would also increase further into the temperate region (Poynton 1962). Thus, a combination of species inhabiting the central interior of the region coupled with relict populations from each centre resulting from these climate driven contractions and expansions into the higher altitude central interior of the region, gave rise to what Poynton termed the 'transitional' fauna. This further suggests that the species composition of the transitional fauna is very heterogeneous (Poynton 1962). Thus, given the heterogenous nature of this transitional assemblage and the direct impact of climate in influencing its species composition and species interactions throughout time; it is rather surprising that this fauna has not been a focus of paleoclimatic studies using new techniques such as species distribution models (SDMs).

Advances in Geographic Information Systems (GIS), paired with the availability of large scale climate data at different climatic periods has provided us with the opportunity to test Poynton's hypothesis regarding the influence of climate on the distribution of sub-Saharan anuran species (e.g., Schreiner et al. 2013). Earlier work dedicated towards understanding the impact of climate change conducted on southern African species focused largely on plants of the Cape Floristic Region (Hannah et al. 2002; Midgley et al. 2002; Rutherford et al. 1999). Studies conducted on the impact of climate change on anurans have largely been limited to the changes in the last 100 years (Botts et al. 2011; Botts et al. 2012; Botts et al. 2013; Botts et al. 2015, but see also Garcia et al. 2014). In addition to climate change, habitat modification (leading to more fragmented distributions) has also been highlighted as an important factor affecting anuran species distributions in the region (Botts et al. 2013; Hof et al. 2011). The general consensus from these correlation-based SDMs is that the South African fauna is expected to shift eastward and both east and southward movement for flora in the south-western Cape (Erasmus et al. 2002; Rutherford et al. 1999). Moreover, species are expected to lose climatic suitability, which will lead to an increase in the spatial mismatch with the current conservation portfolio (Coetzee et al.

2009). However, these results suggest that species will have singular responses to any climate change scenario despite the differences in species' ecologies (Bellard et al. 2012; Thuiller et al. 2006a).

To assess the predicted impacts of climate change on vertebrate ectotherms, correlation based Species Distribution Models (SDMs) have been conducted on endemic or threatened species for Europe (Araújo et al. 2006) and South-east Asia (Bickford et al. 2010) and sub-Saharan Africa (Garcia et al. 2014). Studies conducted in South Africa have also focused on narrowly distributed endemics (Mokhatla et al. 2015; Schreiner et al. 2013). On the contrary, widely distributed species have largely been studied from a biological invasion perspective: i) the American bull frog, (*Lithobates catesbeianus*: Giovanelli et al. 2008), ii) Cane toad (*Rhinella marina*: Urban et al. 2007) and iii) the African clawed frog (*Xenopus laevis*: Ihlow et al. 2016; Measey et al. 2012; Rödder et al. 2017), with few focusing on climate refugia (although see Carnaval et al. 2009). In addition to understanding the impact of climate change on the biotic velocity of species (Ordonez & Williams 2013), it is also important to understand how these climatic changes have structured species distributions from a landscape fragmentation point of view (see Chapter 2, Duan et al. 2016), seeing that amphibians have highly structured genetic populations, even for widely distributed species (Tolley et al. 2010).

The aim of this study is thus to determine the impact of climate change on the distribution of three widely distributed, temperate African anuran species with different ecological specialisations: the principally-aquatic African clawed frog (*Xenopus laevis*), stream-breeding common river frog (*Amietia delalandii*), and the largely terrestrial raucous toad (*Sclerophrys capensis*). Using correlative SDMs (see chapter 2), the aim of the study is to assess the impact of climate change on the distributions of the three-species considered. Firstly, the aim of this study was to determine how changes in climate have influenced species distribution shifts since the Last Glacial Maximum (LGM) through to current distributions and how these will be affected by the 2080. Secondly, I wanted to estimate how distribution range sizes are likely to change as a function of climate. Thirdly, to determine how fragmentation affects frog distributions as climates change. Lastly, using different general circulation models (GCMs), the aim was to understand the role played by climatic variability on estimates of extinction threat (e.g., (Thuiller 2004). Previous studies have suggested that species in southern Africa are expected to shift their distributions on both north and eastward directions (see Chapter 1, see also Erasmus et al. 2002). Furthermore, Botts et al. (2015) suggeted that anuran species in the region (predominantly the transition fauna) have already shifted to higher altitude areas as well as in a north-westerly direction, particularly in the last 100 years.

Methods

Species

I chose three widely distributed yet sympatric species to test the generality of the trends observed in Chapter 2. In addition to being ecologically distinct, these species cover different biomes (e.g., Grassland, Savanna, Fynbos, Nama Karoo, Indian Ocean Coastal belt and the Sacculent Karoo) as well as rainfall regimes (summer, all year and winter rainfall areas). These species are also robust, surviving for extended periods in the laboratory (see Chapter four and five). See chapter three for the detailed differences in histories.

Occurrence data

I used the most recent taxonomic treatment of the target species (Frost 2017) especially as two of the three species have recently undergone taxonomic revisions (Channing et al. 2016; Ohler & Dubois 2016; although see Furman et al. 2015 for *X. laevis*). Occurrence records were obtained from the South African Frog Atlas Project (SAFAP: Minter et al. 2004), CapeNature, I-Spot, published literature (e.g., De Busschere et al. 2016; Ihlow et al. 2016; Measey & Channing 2003) including records from several South African Natural History museums and G.J Measey's *X. laevis* distribution records. I only incorporated records with accuracy to the nearest second or higher. For *Xenopus laevis* I used 789 records, *A. delalandii* 1038 records and *S. capensis* 728 records. Distribution records were projected onto Arc Map GIS 10.3 (ESRI 2014) to identify any doubtful records that may be recorded outside known ranges, using Global Amphibian Assessment (GAA 2008, IUCN; http://www.iucnredlist.org/ accessed July 2017) polygons as 'know distribution ranges'. These were used to judge whether the outlying records represented credible sightings or should be excluded from further analyses.

Climate data

See Chapter two for details.

Species Distribution Modelling

Species distribution model projections were created at a 2.5 arc minute resolution, using current distribution data to reconstruct hypothetical distributions for paleo- and future climate. To reconstruct past distributions, I projected SDMs onto paleo-climate for the Last Glacial Maximum (LGM ≈ 21 Kya). I used three GCMs: the Community Climate System Model (CCSM: Otto-Bliesner et al. 2006), the Model for Interdisciplinary Research on Climate (MIROC: Hasumi & Emori 2004), and the Max-Planck Institut für Meteorologie (MPI-ESM-P: Giorgetta et al. 2013). Paleo-climate data were downscaled to a spatial resolution of 2.5 arc minutes following the delta method (Peterson & Nyari 2008). Current distributions were based on SDM projections onto mean climate from 1950 to 2000. To

forecast future distributions for the year 2080, I used similar GCMs to construct future correlative models under the RCP 8.5 climate scenario which represents the highest greenhouse emissions, while assuming high population growth, slow income growth, and modest technological advances (Riahi et al. 2011). These future GCMs included the CCSM4 (Gent et al. 2011), MIROC-ESM (Watanabe et al. 2011) and the MPI-ESM-LR (Block & Mauritsen 2013).

The selected environmental variables were used to model both past and future potential distributions of anuran species using an ensemble species distribution modelling framework, BIOMOD2 (Thuiller et al. 2013), a multi-model platform implemented in R (R Development Core Team 2017). The BIOMOD2 procedure uses an ensemble of modelling techniques including Generalized Linear Models (GLM), General Additive Models, Generalized Boosted Models, Classification Tree Analysis, Artificial Neural Networks, Surface Range Envelope, Flexible Discriminant Analysis, Multi Adaptive Regression Splines, Random Forests, including Maximum Entropy to produce consensus models, which are weighted averages between all models (Marmion et al. 2009). To ensure that the models were comparable across scale, I used a binomial GLM to rescale the models.

I randomly selected 1000 pseudo absence records, three times within a circular buffer of 100 km enclosing the species records as environmental background. I divided the species records into 80% used to train the models and 20% for model evaluation using three different performance criteria (area under the receiver operating characteristic curve: AUC Swets 1988), true skills statistics (TSS) and Cohen's Kappa (Allouche et al. 2006). As a quality threshold for the final ensemble, I selected only those models with an AUC score > 0.7. The final ensemble was built using a proportional weighting of the models according to their predictive performance. As a presence-absence threshold, I selected the maximum sensitivity and specificity (*max SSS*) training presence as non-fixed threshold (Liu et al. 2005; Liu et al. 2016; Liu et al. 2013). See chapter two for detailed methods.

Statistical analysis

To assess changes in species distributions across climatic periods, I used the binary prediction outputs as the final models. I then determined the level of overlap between distribution ranges across time, with the LGM distributions as the baseline by comparing the Schoener's *D* (dismo package: Hijmans et al. 2017) implemented in R (R Development Core Team 2017). To explore how individual species distributions change across time, I determined the species centroids using the 'sp'package (Pebesma & Bivand 2005; see also Duan et al. 2016 for similar methods). The distance between the centoids was used to determine biotic velocity between different time periods (see chapter 1). Using PatchStat function from the SDMTools package (VanDerWal et al. 2014), I calculated the total area, perimeter and shape index to determine the fragmentation index across time (i.e., Landscape Dissection Index or

shape index, DI´ following Bowen & Burgess 1981). This was achieved by determining the edge of each species distribution range, in relation to the core of the distribution using the formula:

$$DI' = \sum_{i=1}^{n} P/(2\sqrt{\pi \sum_{i=1}^{n} A})$$

where DI´ is Landscape Dissection Index, *P* is Perimeter and *A* is the Area; DI´ compares the amount by which any "island" or a distribution range is dissected relative to the total area, with a DI´ of 1 representing a circle, suggesting a less fragmented shape with a low Perimeter to Area ratio.

Results

Model performance and variables importance

The ensemble models performed very well for the three-distinct species: *A. delalandii* (Cohen's Kappa = 0.687; TSS = 0.805; ROC = 0.954), *S. capensis* (Cohen's Kappa = 0.641; TSS = 0.75; ROC = 0.941) and *X. laevis* (Cohen's Kappa = 0.613; TSS = 0.788; ROC = 0.938). I found that BIO17 (precipitation of driest quarter: 21.37%) was the most important environmental factor limiting the distribution of *A. delalandii*. For *S. capensis*, BIO19 (precipitation of coldest quarter: 26.65%) came out as the most important variable while BIO15 (precipitation seasonality: 22.34%) was identified as the most important environmental variable limiting the distribution of *X. laevis* (Table 3.1). Four environmental variables were found to have variable contributions below 5% for *A. delalandii*, while *S. capensis* and *X. laevis* had only two variables that contributed less than 5% (Table 3.1).

Table 3.1. Variable importance: Percentage contribution of final set of bioclimatic predictors used after determining the inter-correlation structure of the initial 19 variables.

		Variable Contribution (%)		
ID	Bioclimatic variable	*Amietia delalandii*	*Sclerophrys capensis*	*Xenopus laevis*
BIO10	Mean temperature of warmest quarter	10.65	18.59	12.39
BIO11	Mean temperature of coldest quarter	8.20	13.56	12.06
BIO13	Precipitation of wettest month	13.38	7.28	4.19
BIO15	Precipitation seasonality	20.26	6.78	22.34
BIO17	Precipitation of driest quarter	21.37	8.71	19.81
BIO19	Precipitation of coldest quarter	4.68	26.65	5.66
BIO3	Isothermality	2.78	2.80	5.44
BIO7	Temperature annual range	3.04	6.71	4.46
BIO8	Mean temperature of wettest quarter	4.06	5.60	7.04
BIO9	Mean temperature of driest quarter	11.57	3.32	6.60

Range shifts

Models suggest that the predicted niche space occupied by the three species since the LGM to current distributions has not changed significantly between LGM and current conditions (Table 3.2), although more changes are expected between current distributions and 2080 for all GCMs (see also Figure 1A-C), particularly for *A. delalandii* and *S. capensis*.

Table 3.2. Niche overlap calculated as Schoener's *D* between baseline different time periods, using the LGM distribution as baseline. Numbers closer to one represents little or no change in niche space.

	Amietia delalandii	*Sclerophrys capensis*	*Xenopus laevis*
CCSM LGM – Current	0.95	0.92	0.93
Current – CCSM 2080	0.92	0.89	0.96
MIROC LGM – Current	0.96	0.93	0.94
Current – MIROC 2080	0.90	0.88	0.88
ESM-MPI LGM – Current	0.96	0.93	0.94
Current – ESM-MPI 2080	0.90	0.90	0.95

The similarity in niche space between current and future conditions (2080) for *X. laevis* might be slightly better compared to niche space similarities between LGM and current conditions (Table 3.2), under CCSM and EMP-ESM circulation models. The rate at which species tracked climatic changes between the LGM and current distributions is very low compared to the predicted changes by the year 2080. *Amietia delalandii* shifted its distribution southward between LGM and current for all the different GCMs at the rate of 0.003 km.yr^{-1}. *Sclerophrys capensis* repositioned its distribution in a southwestern direction between the LGM and current conditions at the rate of 0.01 km.yr^{-1} under all GCMs considered. To keep up with climatic changes between LGM and current conditions, *X. laevis* shifted its distribution range in a southwestern direction at the rate of 0.001 km.yr^{-1} under CCSM and 0.01 km.yr^{-1} under both MIROC and MPI-ESM CGMs (Figure 3.2).

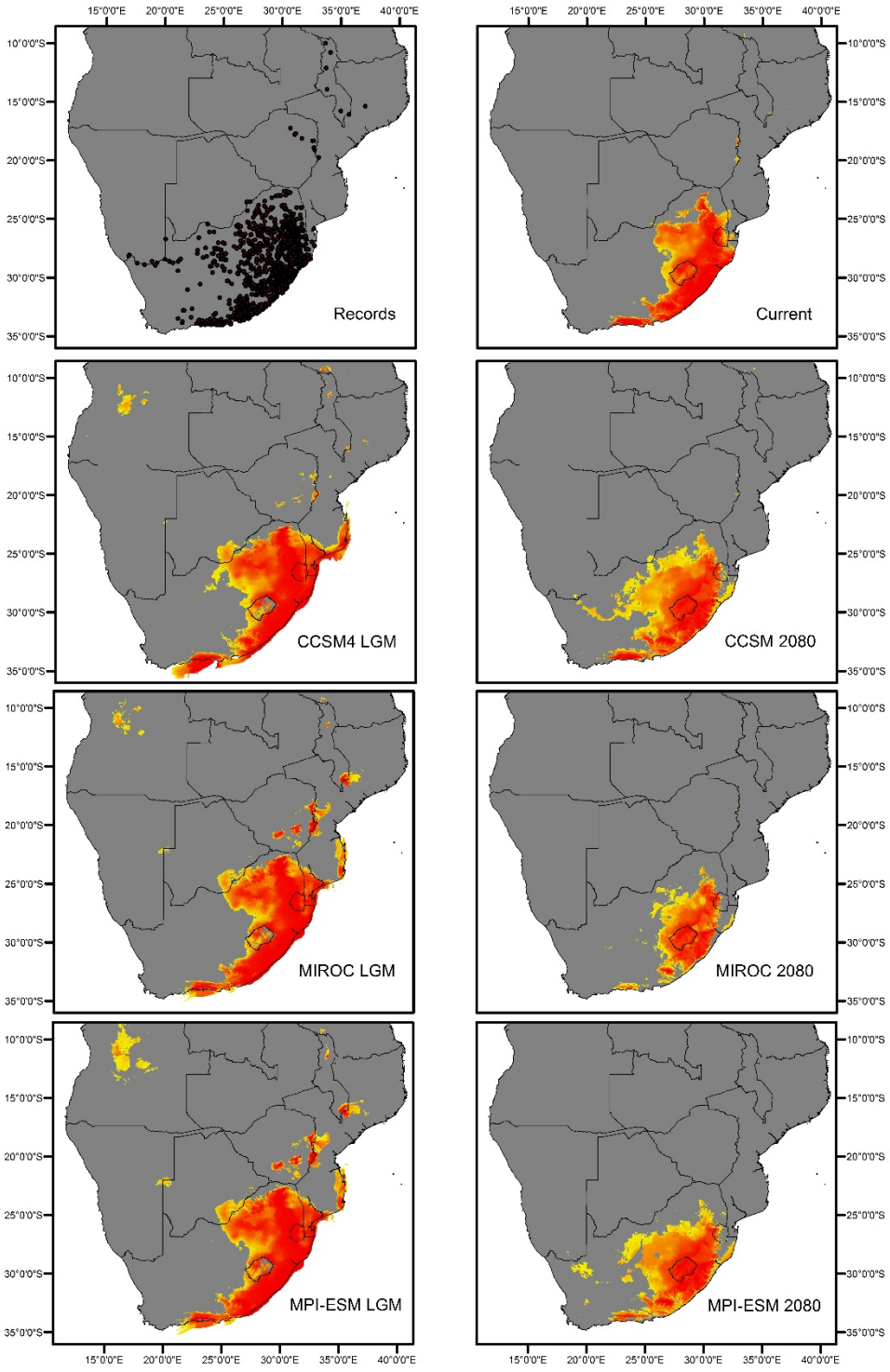

Figure 3.1 A. *Amietia delalandii* distribution records and correlative SDM based projections from the last glacial maximum, current through to predicted projection for the year 2080 using different GCMs.

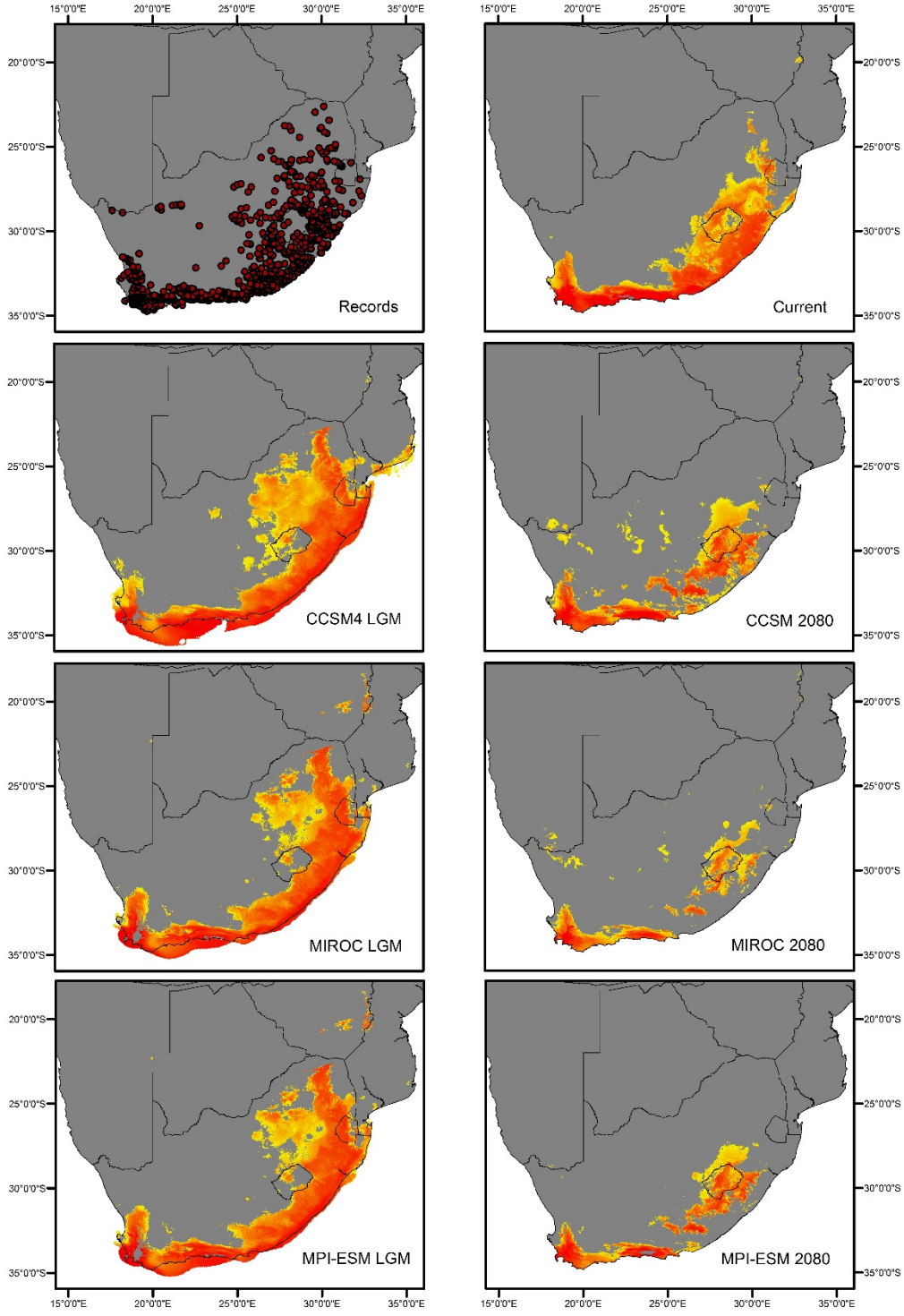

Figure 3.1 B. *Sclerophrys capensis* distribution records and correlative SDM based projections from the last glacial maximum, current through to predicted projection for the year 2080 using different GCMs.

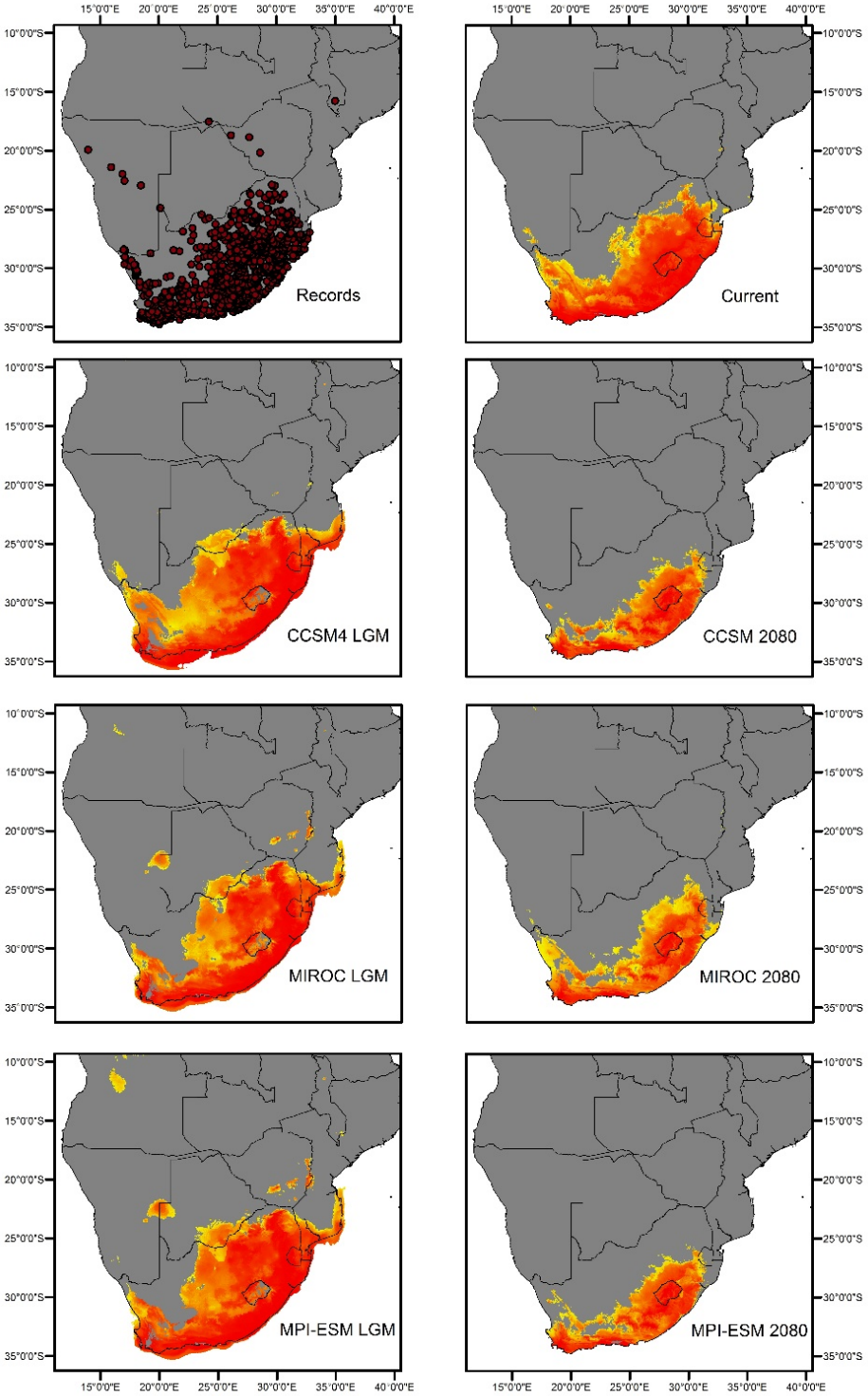

Figure 3.1 C. *Xenopus laevis* distribution records and correlative SDM based projections from the last glacial maximum, current through to predicted projection for the year 2080 using different GCMs

However, in order to keep up with predicted climate change between current and future conditions (2080), *Amietia delalandii* is expected to shift its distribution range in a southwestern direction at the rate of 1.76 km.yr^{-1} (CCSM) and 1.63 km.yr^{-1} (MPI-ESM). For MIROC 2080, the distribution is expected to move southwards between now and the year 2080, at the rate of 0.23 km.yr^{-1} to keep up with climate change. *Sclerophrys capensis* is expected to reposition its distribution range toward the northeast at the rate of 2.53 km.yr^{-1} (CCSM) and 2.59 km.yr^{-1} (MPI-ESM) and in a southwestern direction at the rate of 8.21 km.yr^{-1} under MIROC GCM. *Xenopus laevis* is expected to move southwards for all GCMs considered at the rate of 1.67 km.yr^{-1} (CCSM), 0.79 km.yr^{-1} (MIROC) and 1.79 km.yr^{-1} (MPI-ESM; see Figure 3.2).

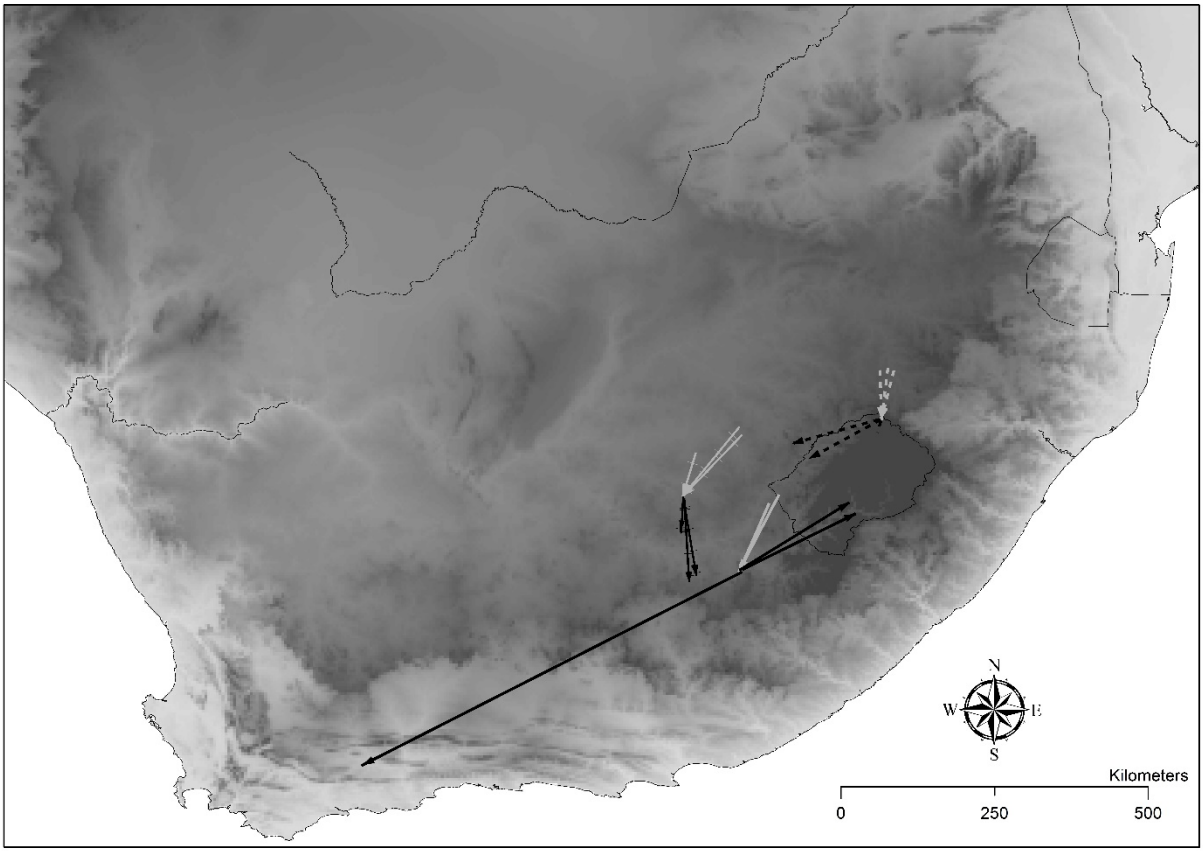

Figure 3.2. Predicted changes in distribution ranges (biotic velocity) across different climatic periods using the different GCMs. The arrows represent distance and direction of species range shifts at different climatic periods, grey arrows represent predicted biotic velocity between the LGM and Current, black arrows represents predicted biotic velocity between Current and 2080. Dashed arrows represent *Amietia delalandii*, solid arrows represent *Sclerophrys capensis* and marked arrows represents *Xenopus laevis*.

Changes in area of occupancy

I found that all species lost suitable climate space between the LGM and current conditions, across all GCMs (Figure 3.3). Between current and future conditions, *S. capensis* and *X. laevis* are expected to lose more suitable climate space for all GCMs considered (Figure 3.3 B and C). More specifically, between LGM and current conditions, models suggest that *S. capensis* lost 31 to 39%, *X. laevis* lost between 27 to 35%, while *A. delalandii* lost between 36 and 44% of suitable climate space across all circulation models. Between current and future conditions (2080), models suggest that *A. delalandii* is expected to lose 28.5% of suitable climate space only under MIROC GCM. However, under CCSM and MPI-ESM GCMs, models predict that *A. delalandii* will gain suitable climate space by approximately 25% and 7% for both GCMs respectively (Figure 3.3A). Both *S. capensis* and *X. laevis* are expected to lose suitable climate space between current and future conditions for all GCMs: CCSM (23.92% and 44.49%), MIROC (54.42% and 28.72%) and MPI-ESM (51.50% and 46.03%) respectively.

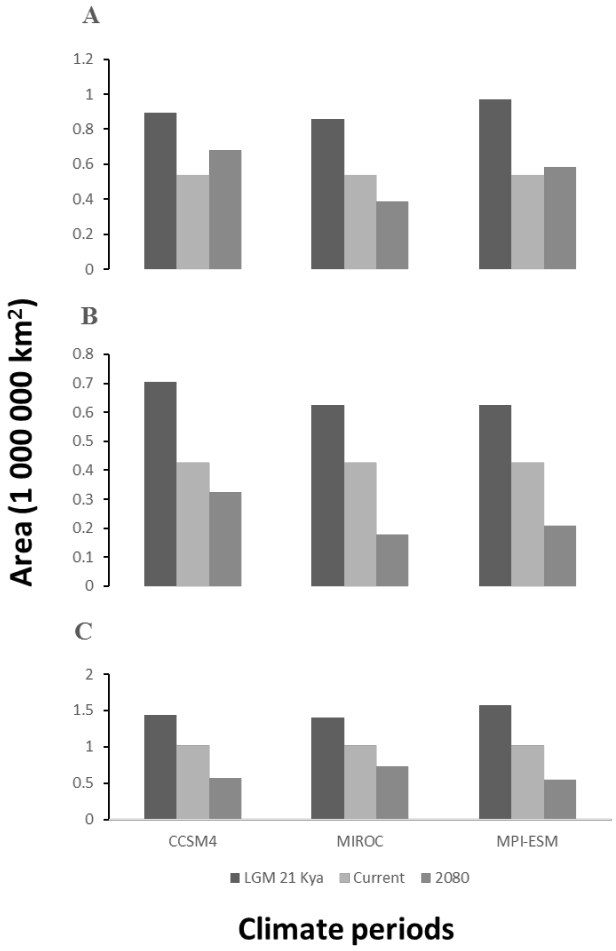

Figure 3.3. Predicted changes in distribution range sizes using different GCMs. A = *Amietia delalandii*; B = *Sclerophrys capensis*; and C = *Xenopus laevis*, based on correlative based SDMs.

***Landscape dissection Index* (DI')**

The models predict that the landscape dissection index (DI') decreased (less fragmented) between LGM and current conditions for all GCMs considered (Figure 3.4). Between current and future condition, DI' is expected to increase for both *A. delalandii* and *S. capensis* for all GCMs. For *X. laevis* DI' is expected to increase slightly under the CCSM, decrease under MIROC and MPI-ESM GCMs (Figure 3.4).

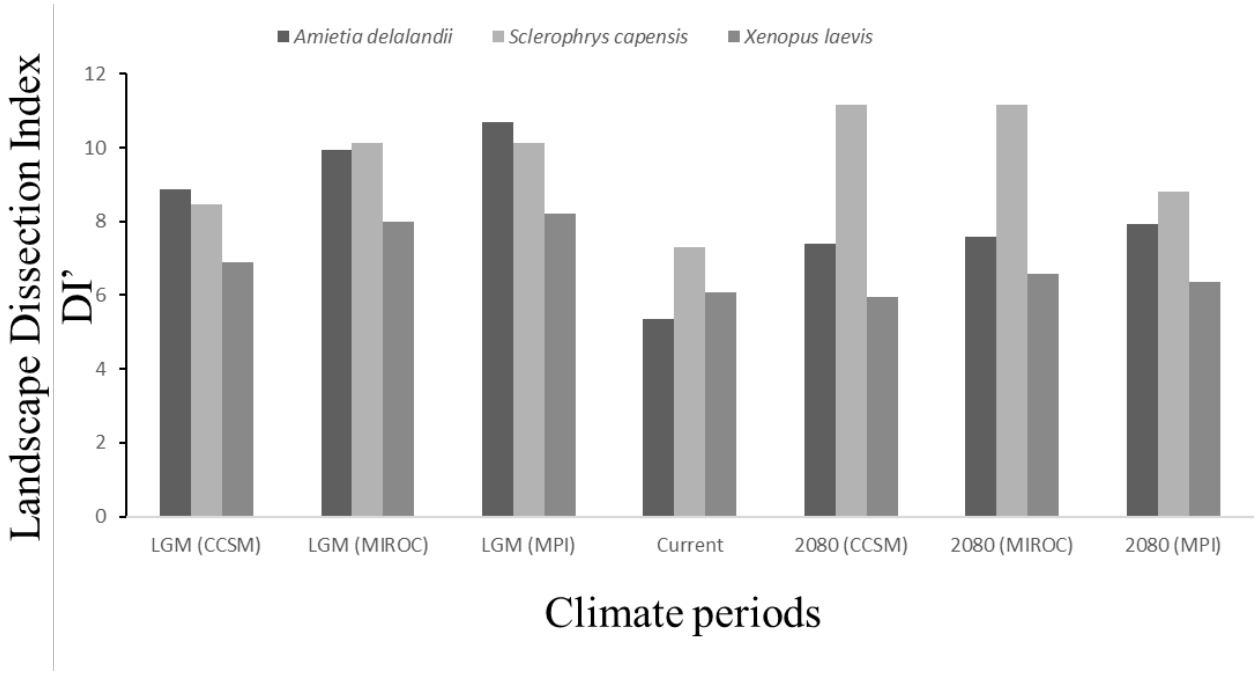

Figure 3.4. Changes in species Landscape dissection index (DI') across climatic periods. Black bars = *Amietia delalandii*; Grey bars = *Sclerophrys capensis* and Dark grey bars = *Xenopus laevis*.

Discussion

The results of this study suggest that although widely distributed anuran species that characterise the transitional fauna as described by Poynton (1960) may have shown similar responses to climatic changes in the past, current human induced climate change might lead to a variation in how species respond through changes in their distributions. Firstly, the results of the correlative based SDMs suggest that the three-transitional species considered in this study have experienced significant range contractions between LGM and current conditions, across all GCMs, although *A. delalandii* is expected to gain suitable climate space between current and future conditions, at least under CCSM and MPI-ESM general circulation models. Secondly, the rate at which species move in keeping up with suitable climatic conditions is expected to be significantly high between current and 2080 conditions as compared to the rate at which species moved between LGM and current conditions. However, I am of

the view that given the unstable climate history characterising the transitional fauna (Poynton 1962), characteristically large distribution ranges (see van Bocxlear et al. 2010) and performance abilities of these species (see chapter five), one would expect these species to keep up with predicted changes in climate. However, this may be greatly impacted by the extent of habitat fragmentation in the region, particularly in the last 100 years (Botts et al. 2015). Thirdly, the results of this study further suggest that the rate of habitat fragmentation is also expected to increase between current and future conditions.

To deal with the impacts of climate change, individual species are expected to change behaviourally, physiologically, genetically, move to climatically suitable habitats if they cannot cope with prevailing conditions or go extinct (Jump & Penuelas 2005). The results suggest different species will show different directional shifts to changes in climate (see Figure 3.3 and 3.4). In a multi-taxon study conducted in the UK, Hickling et al. (2006) found that different species responded differently to changes in climate such that the majority of vertebrate and invertebrate taxa shifted northwards, with reptiles and amphibians showing a significant southward shift. After assuming unlimited dispersal, Araújo et al. (2006) concluded that only 5% of European amphibians were expected to lose suitable climate space, while 26% were expected to gain suitable climate space by the year 2050. However, after assuming that there would be no dispersal, all amphibian species were expected to lose suitable climate space (Araújo et al. 2006).

Evidence suggests that species showed differential responses to climatic changes though time, largely because of differences in species-specific traits (e.g., differences in functional groups or physiology; see Davis & Shaw 2001). While studying the responses of endemic plant species in Namibia, Thuiller et al. (2006b) concluded that different species showed varying responses to climate change after partitioning the species into different biological types, supporting a functional group response. Studies focusing on endemic species (e.g., anurans, mammals, birds, reptiles, insects and plants) in southern Africa have concluded that these species are expected to show similar responses to expected changes in climate. For instance, many studies have concluded that endemic species in the region (irrespective of taxa), are expected to shift eastward as well as losing suitable climate space as a result of climate change (Erasmus et al. 2002; Hannah et al. 2007; Hannah et al. 2005; Hannah et al. 2002; Mokhatla et al. 2015).

Two of the three GCMs considered in this study suggest that *A. delalandii* is predicted to gain suitable climate space under future climatic conditions. A combination of factors may help to put this result into context. First, although climate models generally predict a drying trend in the south-western Cape and the north-eastern parts of southern Africa (e.g., Hoerling et al. 2006; Shongwe et al. 2009), Engelbrecht et al. (2011) further predicted an increase in rainfall, particularly central interior of the region. This predicted increase in rainfall might make conditions suitable for *A. delalandii* to expand its distribution into these areas. Secondly, the ability to cope with higher environmental temperatures may not be as

limited as predicted such that widely distributed species such as *A. delalandii* may be currently occupying environments that are well below its critical thermal maximum (Araújo et al. 2006; Snyder & Weathers 1975; Sunday et al. 2011). Indeed, in ectotherms, environmental temperature does set limits to key physiological processes contributing to species distributions (see chapter four and five). Third, because large distribution ranges and low endemic richness are a response of unstable historical climates (e.g., see Araújo et al. 2008 regions that may have experienced fluctuating climates such as the transitional fauna), it is not surprising that, under certain conditions, widely distributed species such as *A. delalandii* are expected to gain suitable climate space (see Dynesius & Jansson 2000). This indeed suggest that such species may tap into their history to cope with challenges posed by a warming world, provided that current land-use changes do not prove detrimental to predicted climate shifts. Lastly, perhaps a combination of (increased) surface water availability and the effect of temperature on desiccation tolerance may be setting limits to the distribution of *A. delalandii*, not temperature per se (see chapter six).

The result of this study supports similar species responses only between LGM and current conditions. Furthermore, recent evidence also suggests that methodological differences may account for a large proportion of reported species range shifts as opposed to biological traits driven range shifts (Brown et al. 2016; see also Fei et al. 2017). Studies focusing on endemic species may be reporting on the general trend as opposed to focusing on different species-specific responses (see chapter two). Moreover, we may not fully understand how sustained extreme climate events such as draughts may affect species responses. Perhaps, there could be oscillating distributions associated with these wet/dry periods which may lead to more fragmented distributions and rely on hubs of metapopulation dynamics. In addition, endemic species may perhaps be expected to show similar directional responses to climate change as most evolved under relatively stable climatic regimes and may thus share similar climate history (Dynesius & Jansson 2000). In contrast, widely distributed species may have experienced different climatic conditions and may thus be driven by different climatic influences (e.g., the different transitional fauna by Poynton 1964) as suggested by possible gain in suitable climate space for *A. delalandii*.

Perhaps the biggest challenge for the transitional anuran fauna of southern Africa is not the emergence of non-analogue biotic interaction (see Poynton 1964, Alexander et al. 2004), but rather the loss of suitable climate space habitat and increased fragmentation. Although this result provides a good platform for evaluating the effect of climate change on widely distributed anuran species in the region, caution should be exercised when interpreting these results. First, predicted ranges loses/gains may be difficult to dissociate from those driven by changes in land-use, particularly in southern Africa where both factors are expected to act in synergy, particularly on anuran species distributions (Botts et al. 2015; Hof et al. 2011). Indeed, several species such as *X. laevis* and the painted reed frog (*Hyperolius marmoratus*) have experienced range expansions, particularly in the last 100 years because of habitat

modification (Davies et al. 2013, Botts et al. 2015, Measey et al. 2017). Although correlative based SDMs are important in conservation and ecology, the climatic variables which are used as inputs to predict distributions are recorded at coarse spatial resolutions and may thus not reflect the conditions that are experienced by lower vertebrates such as anurans, leading to flawed species distributions. Lastly, in addition to environmental variables, factors such as biological interactions play an additional important role in determining the realised niche of each species (Ensslin et al. 2017; Suttle et al. 2007).

In conclusion, this study provides support for the idea that although past climatic changes may have led to similar species responses, current human induced climatic changes may lead to differential (species-specific) responses. This is particularly important given the unstable historical climate characteristic of the transitional fauna, as opposed to the stable historical climate of the CFR (chapter two). Perhaps this unstable climate history may give rise to the differential species responses observed in this study (i.e., predicted increase in suitable climate space for *A. delalandii*). I believe that the time is ripe for investigations into the possible role that climate played in structuring anuran species distributions in this transitional region on a full suite of transitional species. This may assist in understanding future impacts of climate change, while contrasting different regions that experienced different historical climates. In addition to considering differences in species biology, future studies should consider incorporating climate variability into the modelling process. Moreover, future studies should look into incorporating biological traits such as physiology and performance into SDMs so as to capture these inherent biological differences into species modelling processes through the use of mechanistic models (Kearney et al. 2008; Kearney & Porter 2009; Kearney & Porter 2004)

Chapter four: The role of ambient temperature and body mass on body temperature, standard metabolic rate and evaporative water loss in southern African anurans with different ecologies†

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Abstract

Temperature and water availability are two of the most important variables affecting all aspects of an anuran's key physiological processes such as body temperature (T_b), standard metabolic rate (SMR) and rate of evaporative water loss (EWL). Since anurans display pronounced sexual dimorphism, evidence suggest that differences in SMR and EWL are further influenced by other factors such as sex and body mass (M_b). However, few studies have tested the generality of comparing species with different life histories across a wide range of available ambient temperatures (T_a), while taking anuran species ecology into account. Thus, using three species with different ecological specialisation: the principally-aquatic African clawed frog (*Xenopus laevis*), stream-breeding common river frog (*Amietia delalandii*), and the largely terrestrial raucous toad (*Sclerophrys capensis*), the aim of this study was to determine the role of T_a on T_b, whole-animal SMR and whole-animal EWL in these wild caught African anuran species. Experiments were conducted at a range of test temperatures (5°-35°C, at 5°C increments). We found that T_b, whole-animal EWL and whole-animal SMR increased with an increase in temperature. Body temperature increased with an increase in M_b in *A. delalandii* and *S. capensis* but not in *X. laevis*. We did not find any significant effect of both M_b and sex on whole-animal EWL in all three species. Whole-animal SMR increased with an increase in M_b in *S. capensis* only. Mass-specific EWL and SMR decreased with an increase in M_b. Although we found that T_a is very important in influencing key physiological processes, the generality of M_b as an important factor should be taken in the context of species ecology, particularly in sexually dimorphic taxa such as amphibians.

Introduction

Water and temperature are the two most important ecological determinants of species distribution patterns (Buckley & Jetz 2007; Qian et al. 2007) through their influence on environmental energy availability (Chown et al. 2003; Currie 1991). At a landscape level, they influence species abundance as well as activity patterns (Dabés et al. 2012; Peterman & Semlitsch 2014). At the physiological level, animals constantly need to maintain a positive energy and water balance in order to meet their short and long-term energy requirements for growth, development and reproduction (McNab 2002). In contrast to endotherms (most birds and mammals; see Geiser 1998), regulation of body temperature (T_b) of ectotherms is external (i.e., their T_b is driven by the environmental temperature of their habitat) and has been found to be highly correlated with ambient temperature (T_a : Angilletta et al. 2002; Brattstrom 1979; Huey 1991). In vertebrate ectotherms (fish, amphibians and reptiles), the regulation of T_b is maintained largely through behavioural means by selecting suitable microclimates (Brattstrom 1979; Herrel & Bonneaud 2012; Herrel et al. 2014; Köhler et al. 2011; Lillywhite 1970; Seebacher & Alford 2002; Wilson et al. 2000). Thus, given the diverse array of life-history traits within anurans and associated microclimates we might expect a variation in experienced T_b, even within the same biogeographic setting.

The maintenance of optimal T_b, through the selection of suitable microclimate sites, determines how ectotherms interact with their environment. Therefore, several processes vital for survival such as food assimilation rates, performance and ultimately fitness; are all temperature dependent (Angilletta 2001; Angilletta et al. 2002; Buckley et al. 2012; Huey & Kingsolver 1989; Huey & Stevenson 1979; Seebacher & Franklin 2005). Furthermore, T_b is crucial in determining key physiological processes such as evaporative water loss (EWL) and standard metabolic rates (SMR) in vertebrates (Huey 1991); such that both EWL and SMR increase with an increase in temperature (Gillooly et al. 2001). This is mainly because higher temperatures generally increase enzyme reaction rates (Brown et al. 2004; Gillooly et al. 2001).

Moreover, these coupled increases in SMR and EWL are known to be affected by body mass (M_b) such that large bodied individuals have relatively low mass-specific rates of metabolism and water loss, irrespective of taxon (Gillooly et al. 2001). In addition to the role of M_b in determining rates of EWL and SMR, several studies suggest that the ecology of an organism can drive EWL requirements (Thorson 1955). Evaporative water loss is particularly pronounced in amphibians because most have moist, highly permeable skin (Shoemaker & Nagy 1977; Spotila & Berman 1976). Indeed, most amphibians lack physiological adaptations to regulate water loss; thus suggesting that the rate at which some amphibians lose water is similar to an open water-body of a similar size (Spotila & Berman 1976; Tracy et al. 2007; Wygoda 1984).

However, evidence suggests that amphibians occupying different ecological niches show pronounced differences in rates of EWL with arboreal frogs displaying comparatively reduced rates of cutaneous resistance to water loss as compared to other species groups (Wygoda 1984; Wygoda & Garman 1993; Young et al. 2005; Young et al. 2006). This is achieved by covering their bodies with a water resistant waxy secretion (Barbeau & Lillywhite 2005; Gomez et al. 2006), which increases passive heat loss, whilst reducing evaporative heat loss demands. In contrast, evidence of an ecologically mediated pattern of SMR in ectotherms seems to be lacking since differences in SMR have largely been linked to differences in species activity patterns (Clarke & Johnston 1999).

Understanding the effect of temperature on key physiological traits in different anuran species (with different ecological specialisations) will enable us to better understand how changes in climate will affect this threatened vertebrate group (Pimm et al. 2014). Broader understanding of this temperature *vs* physiology relationship may also provide us with a snapshot of how physiological differences drive species-specific responses of climate change. Climate change is expected to be more pronounced in sub-Saharan Africa with increased drying, particularly in the winter rainfall region and less variable rainfall regimen across the region (Engelbrecht et al. 2013; Giannini et al. 2008) – further placing amphibians of this region at high risk of extinction (Foden et al. 2013; Garcia et al. 2014; Hof et al. 2011; Mokhatla et al. 2015). Southern Africa has a diverse anuran fauna occupying many different habitat types (Alexander et al. 2004), and we may expect variation in the way each species will respond to environmental challenges in their respective environments (Loveridge 1976).

In this study, I determine how ambient temperature affects SMR, EWL and T_b of three different temperate African anuran species. I selected species depending on their apparent dependency on water: the principally-aquatic African clawed frog (*Xenopus laevis*), ii) stream-breeding common river frog (*Amietia delalandii*), and iii) principally-terrestrial raucous toad (*Sclerophrys capensis*). I tested how the variation in T_a affects T_b, whole-animal EWL and SMR and mass-specific EWL and SMR in these same three anuran species. Next, I determine whether these species differ in their responses to T_a fluctuations. I expect that differences in species' response are associated with differences in ecological specialisation. Specifically, I expect aquatic species to show higher EWL compared to terrestrial and semi-aquatic species, as conditions are buffered in aquatic environments (excluding biotic interactions), which may lead to reduced potential for evolutionary adaptation (Toledo & Jared 1993). Because of the relationship between water loss and activity patters (e.g., Peterman & Semlitsch 2014), I hypothesise that terrestrial-adapted species would show reduced metabolic and water loss rates as an adaptation to terrestrial life (e.g., Wygoda, 1984). Likewise, species with intermediate life-histories (closely associated with water but not living in it), would exhibit an intermediary status.

Methods

Study species

I chose three different sympatric anuran species with different ecological specialisations, based on their modes of egg deposition and development (Mercurio et al. 2009). The pipid, *Xenopus laevis*, inhabits and breeds in permanent water bodies. This species is usually referred to as permanently aquatic because it possesses several key physiological adaptations (e.g., lateral line system, webbed hind legs etc.), suitable for an aquatic life style (see Measey 2004). However, evidence also suggests that they can survive on land as observed during overland dispersal episodes (De Villiers & Measey 2017; Measey 2016). *Amietia delalandii* (family: Pyxicephalidae – previously known as *A. angolensis* and *A. quecketti*) breeds in and inhabits streams and flowing rivers. Adults are usually encountered on the water edge and on rocks along streams but are seldom encountered away from water bodies (Channing 2004). *Sclerophrys capensis* (previously known as *Bufo rangeri* and later *Amietophrynus rangeri*) is a member of the toad family Bufonidae. These toads generally breed in shallow, temporary water bodies and adult toads are adapted to a terrestrial mode of life (Cunningham 2004).

Adult individuals of the three-target species were caught around the area of Port Elizabeth, South Africa (Table 4.1). Experiments were undertaken under the animal research ethics clearance permit number A13-SCI-ZOO-007 issued by the Research Ethics Committee (Animal) at the Nelson Mandela University. Animals were collected under permit number CRO41/14CR, issued by the Department of Economic Development, Environmental Affairs and Tourism, Eastern Cape Province. I caught between 20 and 30 individuals (of varying but overlapping interspecific body size classes) excluding juveniles and gravid females. An attempt was made to maintain M_b comparable among species as much as possible, although I found this difficult for *A. delalandii*. I included both male and female individuals to account for variation that might be as result of sexual dimorphism. Sexual dimorphism is ~~a~~ particularly pronounced in anurans, with females generally larger than males (see Table 4.1 for mass differences, with *X. laevis* showing the largest differences between males and females). Animals were kept for a maximum period of 2-4 months in the lab under environmentally enriched conditions (see below), until all experiments were completed. After completing the experiments, animals were returned to their respective sites of capture.

Table 4.1. Sample size and body mass (g) (mean±SD) of individuals used in the experimental trials.

	Male		Female	
Species	**N**	**Mass (g)**	**n**	**Mass (g)**
Amietia delalandii	15	7.65±1.61	9	15.98±6.03
Sclerophrys capensis	10	50.55±8.91	14	82.58±26.26
Xenopus laevis	11	29.87±10.52	22	70.75±30.55

Amietia delalandii were maintained in 110 L plastic boxes, with sand and small logs for cover, at low densities (5 individuals per box). *Sclerophrys capensis* were kept in terraria made from paddling pools (*d x h*: 2.16 m × 0.45 m) with sand, water and small logs and bark to provide cover. *Xenopus laevis* were kept in a fresh water tank (*l x b x h*: 3.55 m × 0.9 m × 0.63 m) with stacked-bricks and stones to provide adequate cover and fed a diet of ox-heart. Both *A. delalandii* and *S. capensis* were fed mealworms and crickets, dusted with calcium (ReptiCalcium, Zoo Med Laboratories Inc; South Africa). All species had food available *ad libitum*, and the holding rooms were maintained at 20°C, on a 12:12 photoperiod. Atmospheric air was circulated to maintain a constant temperature in the holding rooms, thus ambient humidity levels fluctuated with outside air. Following Hillman et al. (2009), feeding of individuals ceased three days prior to experiments to ensure that individuals were post-absorptive during experimental sessions. Water was provided throughout the duration of the study (including periods when food was withheld) to prevent dehydration stress. I further ensured that the terraria in which we held *S. capensis* and *A. delalandii*, were sprayed with water every 3 days to dampen the sand and I provided a bowl with water daily where amphibians could rehydrate. Mass was recorded to the nearest ± 0.01 g before and after each experimental run.

Gas exchange measurements

Standard metabolic rates and evaporative water loss measurements were conducted on the three anuran species following Lighton (2008) and Steyermark et al. (2005), also see Gomes et al. (2004) for a range of respirometry methods used, particularly for ectotherms. I used an open-flow respirometry system operated on a push through mechanism on post-absorptive, non-reproductive individuals, at rest (Sinclair et al. 2013). Experiments were conducted at temperatures ranging from 5°C to 35°C at 5°C intervals (Dunlap 1971). The order of experimental temperatures runs was randomised to reduce the effects of experimental acclimation to any directional shift in temperature. Eight individuals of each species (4 males: 4 females) were randomly selected for trials at each temperature and a single trial was conducted on each individual per temperature.

Prior to each experimental session, frogs were patted dry to remove excess water from the skin and we then recorded M_b to the nearest 0.01 g. All experiments were conducted between 07h00 and 18h00, always within the light cycle of the 12:12 photoperiod, when animals were less likely to be active (Gomes et al. 2004). Frogs were placed individually in suitably-sized air tight glass metabolic chambers of three sizes: 341 ml for small-, 476 ml for medium and 978 ml for large frogs, depending on the size of the individual. I found that individual frogs were agitated when they experienced respirometry procedures for the first time. Prior to the respiromtery experiments, I performed a training session on

each individual frog by placing them inside metabolic chambers for 15 minutes at 20°C. Frogs were noticeably calmer during subsequent respirometry runs.

A 0.5 cm layer of mineral oil was added to each chamber to prevent evaporation of excreted materials. Inside the chamber, a frog was placed on a plastic mesh platform (with sufficiently large holes for faeces to fall through), suspended at least 2 cm above the oil layer (Smit & McKechnie 2010). Air temperatures inside the metabolic chamber were recorded using a thermocouple probe (Fluke 54II*B*, Fluke Corporation, Washington) that was inserted inside the chamber. Once the animal was placed in the chamber, "*cling-wrap*" (GLAD, South Africa) was used before sealing with a glass lid. After placing the lid, I placed *Prestik*, "*Blu-Tac*" type material (Bostik, Cape Town) around the lid of the metabolic chamber to minimise air leaks. Two metabolic chambers, one containing an animal, and the other an empty reference chamber (serving as a chamber to determine baseline levels) were placed in a custom made environmental chamber made from a 100 L cooler box with the interior lined with copper tubing. Baseline levels were recorded for 30 minutes before each trial (Smit & McKechnie 2010; van de Ven et al. 2013). The temperature inside the cooler box was controlled by pumping temperature-controlled water through the copper tubing using a circulating water bath (FRB22D, Lasec, Cape Town, South Africa; see van de Ven, Mzilikazi & McKechnie, 2013). A small fan was used to ensure air circulation inside the environmental chamber.

During SMR and EWL measurements, I used Mass Flow System pumps (MFS-2, Sable Systems, Las Vegas, NV) to pump atmospheric air scrubbed of water vapour (using a Drierite column (98 % $CaSO_4$, 2% $CoCl_2$, Sigma-Aldrich, South Africa) at a flow-rates of 100 to 600 $mL.min^{-1}$, through the metabolic chambers. I calibrated the MFS-2 pumps using a flow-bubble meter (calibrated flow-rates were used in subsequent equations, see below). I scrubbed water vapour from air to have better control of ambient humidity levels in the respiromtery chamber (actual vapour pressures recorded for atmospheric air in Port Elizabeth varied greatly among days during our study period). Air from the metabolic chambers was sequentially sub-sampled, using Subsampler (SS3, Sable Systems, Las Vegas, NV) and a Multiplexer (V3, Sable Systems) was programmed though Expedata (Sable Systems, Las Vegas, NV) to record gas concentrations for each chamber at 20-minute intervals, recording an air sample every second. Subsampled air was first pulled through a water vapour analyser (RH-300, Sable Systems, Las Vegas, NV) to measure water vapour pressure. I was mainly interested in measuring total EWL, not different components of water loss such as boundary layer and cutaneous resistance; hence I did not use agar models in our approach (Buttemer 1990). Air samples then passed through a carbon dioxide analyser (Ca-10a, Sable Systems, Las Vegas, NV) and finally through to an oxygen analyser (Fc-10a, Sable Systems, Las Vegas, NV). The behaviour of the frogs was monitored during the trials using live video feed for the duration of the trial. The thermocouple probe was used to measure T_{air} (air temperature inside the metabolic chamber). During a trial, frogs experienced one controlled temperature

at a time. A trial was considered completed when the water vapour pressure and temperature trace was stable for 20 minutes or if the animal appeared too distressed to continue with measurements. Although I acknowledge that different sized frogs show differences in cooling rates (Wygoda 1988b; Wygoda 1989), I assume that the final 20 minute interval of stable vapour pressure and temperature trace suggest that at this point, each individuals' T_b (irrespective of size) had reached equilibrium with the desired test temperature. Trials did not last longer than 2 hours. After each trial, a frog was removed from the chamber and cloacal temperature (T_b) was recorded within 30s of removal (using a Fluke 80PK-1 probe, Type K thermocouple -40 to 260°C) and the final M_b. After each trial, frogs were individually placed in temporary holding facilities. *Xenopus laevis* were kept in 20 L buckets, half-filled with water at 20° C and fed ox heart. Both *S. capensis* and *A. delalandii* were kept in 0.5 L plastic container lined with a wet lab paper and were fed mealworms and crickets, respectively. After each trial, individuals were eligible for selection for another trial run only after 3 days.

Data extraction

Once all experiments were complete, I used Expedata software to extract oxygen, carbon dioxide and water vapour pressure traces from data files. I selected the most stable 20 minutes trace in each run, when the animal was at rest. Standard metabolic rates can either be calculated using the rate of oxygen consumption or carbon dioxide production rates (Gatten et al. 1992; Lighton 2008; Withers 2001). I used rates of oxygen consumption (VO_2) at each temperature following Gomes et al. (2004). Moreover, flow rate was calibrated using a flow-bubble meter. To determine the rates of EWL, I converted rates of water vapour pressure to water vapour density, which were subsequently converted to rates of EWL (see Lighton, 2008).

Statistical analysis

To determine the effect of T_a on T_b, EWL and SMR of anurans, I used linear mixed-effect models with the R package 'nlme' (Pinheiro et al. 2014) due to repeated measurements on individuals. I also included M_b and sex as fixed effects in models. Furthermore, I wanted to determine if species differed in their responses to changes in T_a. However, this analysis was restricted to species with comparable M_b (i.e., *S. capensis* and *X. laevis*), because it is known to be a significant contributor to observed physiological difference. All analysis were undertaken in R (R Development Core Team 2017).

Results

In ectotherms T_b is significantly correlated to T_a (Angilletta et al. 2002; Brattstrom 1979; Huey 1991). I ran a repeated measures correlation using the R package "rmcorr" (Bakdash & Marusich 2017a; Bakdash & Marusich 2017b). I found a strong, significant positive correlation between the two variables

($r_{(96)}$ = 0.9917; 95% CI (0.988, 0.994), P < 0.001) for all three species. I subsequently replaced T_b with T_a in all ensuing models (see below).

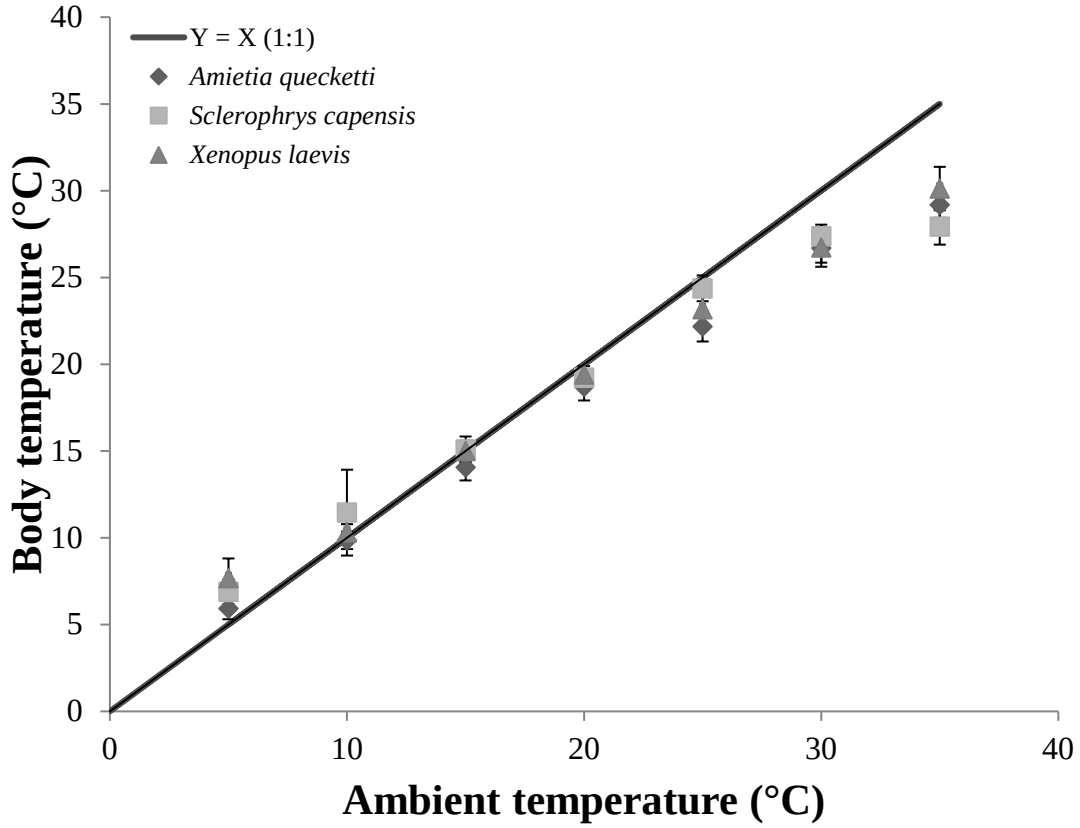

Figure 4.1. Relationship between T_a and T_b of different functional groups over a range of different test ambient temperatures (T_a). The solid line indicates $Y= X$ relationship representing a typical 1:1 T_a *vs* T_b relationship depicting an amphibian incapable of regulating T_b through physiological or behavioural means, to highlight how T_b deviates from T_a particularly at low and high T_a.

African clawed frog: *Xenopus laevis*

I found that T_b was positively correlated to T_a ($t_{1, 26}$ = 53.501; P < 0.0001, see Figure 4.1). I did not find a significant effect of M_b on T_b ($t_{1, 28}$ = 0.459; P = 0.650) and T_b did differ between males and females ($t_{1, 19}$ = 1.149; P = 0.263). Whole-animal EWL for *X. laevis* increased with an increase in T_a ($t_{1, 28}$ = 13.417; P < 0.0001; Figure 4.2A). Body mass did not have a significant effect on both whole-animal EWL ($t_{1, 28}$ = 0.462 P = 0.648) and SMR ($t_{1, 28}$ = 0.103; P = 0.919). Moreover, I did not find any significant differences between males and females for both whole-animal EWL ($t_{1, 19}$ = -1.001; P = 0.327) and SMR ($t_{1, 19}$ = -1.257; P = 0.221). Mass-specific EWL increased with an increase in T_a ($t_{1, 28}$ = 20.071; P < 0.0001) although it decreased with M_b ($t_{1, 28}$ = -9.278; P < 0.0001). Standard metabolic

rate increased with an increase in T_a ($t_{1, 28}$ = 13.828; P < 0.0001; Figure 4.3A). Mass-specific SMR also increased with an increase in T_a ($t_{1, 28}$ = 18.697; P < 0.0001) but decreased with an increase in M_b ($t_{1, 28}$ = -8.846; P < 0.0001). I did not find any significant differences in mass-specific EWL ($t_{1, 19}$ = 0.299; P =0.767) and mass-specific SMR ($t_{1, 19}$ = 0.627; P = 0.537) between males and females.

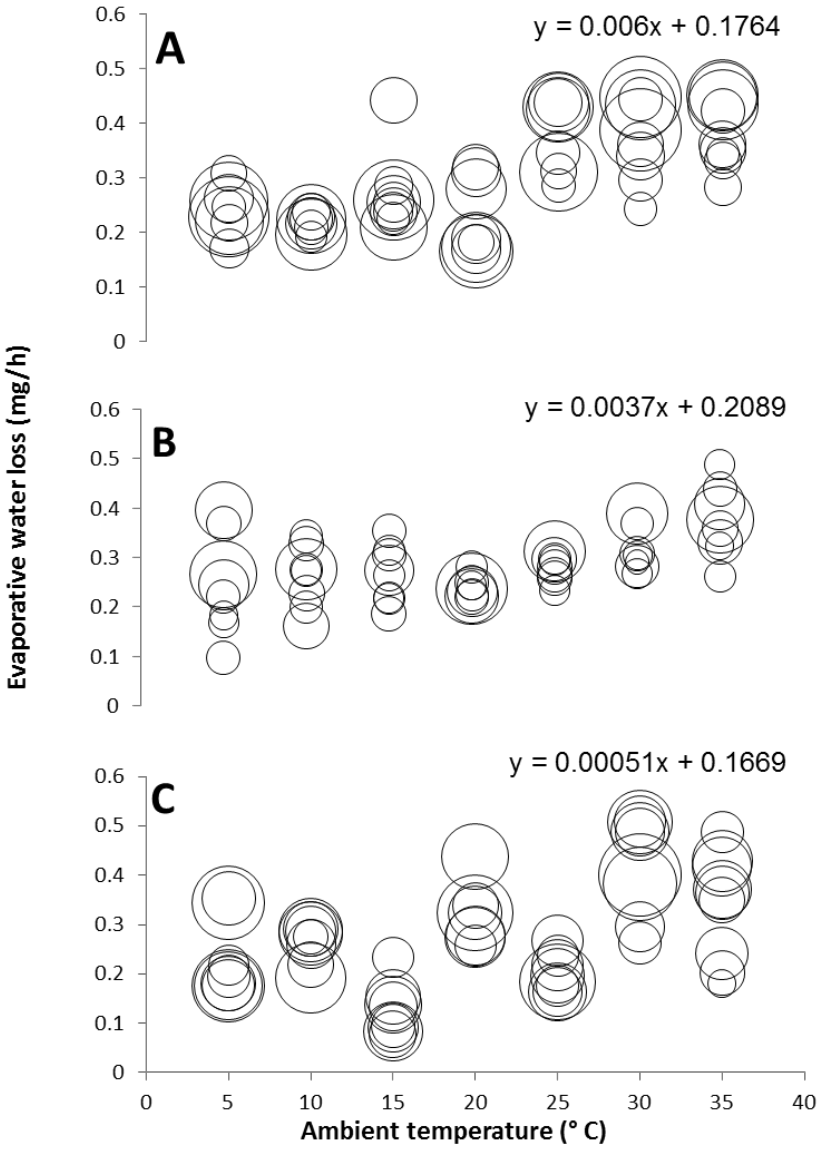

Figure 4.2. Bubble plot showing the relationship between whole-animal EWL and ambient temperature (T_a). The size of the bubbles represent different body sized individuals; larger bubbles represent larger indivuals. We also presented body mass (mean±SE) in grams (g). A. *Xenopus laevis* (54.07 ± 7.09), B. *Amietia delalandii* (12.69 ± 1.71) and C *Sclerophrys capensis* (59.48 ± 7.95).

Common river frog: *Amietia delalandii*

For *A. delalandii*, I found that T_b was positively correlated with T_a ($t_{1, 23}$ = 74.343; P < 0.0001, Figure 4.1). I found that M_b had a significant positive effect on T_b ($t_{1, 28}$ = 2.908; P < 0.05), although I did not find any significant differences in T_b between males and females ($t_{1, 19}$ = -1.086; P = 0.289). Whole-animal EWL ($t_{1, 28}$ = 12.577; P < 0.0001; Figure 4.2B) and SMR ($t_{1, 28}$ = 11.713; P < 0.0001, Figure 4.3B) increased with an increase in T_a. I did not find a significant effect of M_b and both whole-animal EWL ($t_{1, 28}$ = 1.113; P = 0.277) and whole-animal SMR ($t_{1, 28}$ = 1.517; P = 0.143), respectively. Moreover, I did not find any significant differences in both whole-animal EWL ($t_{1, 29}$ = -0.285; P = 0.778) and SMR ($t_{1, 19}$ = -0.383; P = 0.706) between males and females. Mass-specific EWL ($t_{1, 28}$ = 13.035; P < 0.001) and mass-specific SMR ($t_{1, 28}$ = 4.309; P < 0.001) increased with an increase in T_a, but both decreased with an increase in M_b ($t_{1, 28}$ = -4.540; P < 0.001 & $t_{1, 28}$ = -4.417; P < 0.001), respectively (see Figure 4.4 for mass-specific EWL). There were no significant differences in mass-specific EWL ($t_{1, 28}$ = 1.863; P = 0.075) and mass-specific SMR ($t_{1, 19}$ = 1.917; P = 0.067) between males and females.

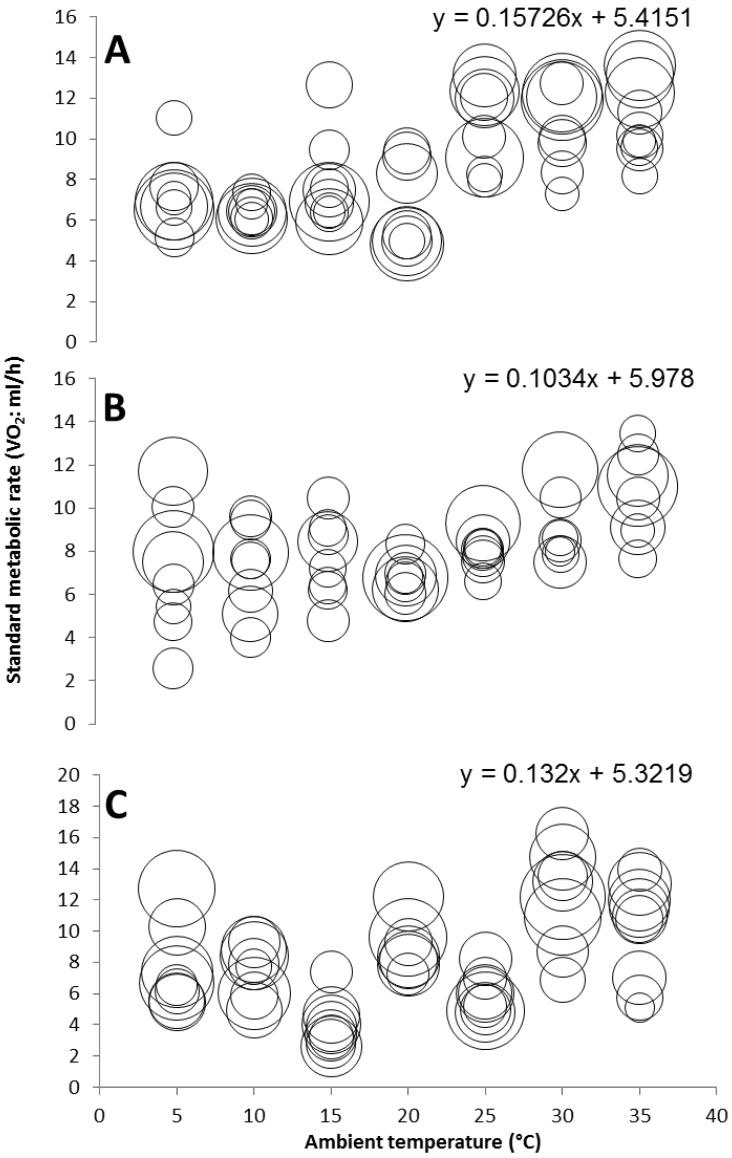

Figure 4.3. Bubble plot showing the relationship between whole-animal SMR and ambient temperature (T_a). The size of the bubbles represent different body sized individuals at each experimental temperature. A. *Xenopus laevis*, B. *Amietia delalandii* and C *Sclerophrys capensis*.

Raucous toad: *Sclerophrys capensis*

Sclerophrys capensis T_b was positively correlated with T_a ($t_{1, 28}$ = 37.007; P < 0.0001, Figure 4.1). I also found that M_b had a significant positive effect on T_b ($t_{1, 34}$ = 2.409; P < 0.05), although I did not find any significant difference in T_b between males and females ($t_{1, 19}$ = -1.398; P = 0.178). Furthermore, I found that an increase in T_a lead to a significant increase in whole-animal EWL ($t_{1, 34}$ = 4.639; P < 0.001; Figure 4.2C). Both M_b ($t_{1, 19}$ = 1.921; P = 0.065) and sex ($t_{1, 19}$ = -1.944; P = 0.067) did not have a significant effect on whole-animal EWL. Mass-specific EWL also increased with an increase in T_a ($t_{1, 33}$ = 13.364; P < 0.0001) but decreased with an increase in M_b ($t_{1, 33}$ = -6.327; P < 0.0001, Figure 4.4).

There were no significant differences in mass-specific EWL between males and females ($t_{1, 19}$ = -0.413; P = 0.684). Whole-animal SMR also increased with an increase in T_a ($t_{1, 33}$ = 6.370; P < 0.0001; Figure 4.3C). In addition, I found that M_b had a positive significant effect on whole-animal SMR ($t_{1, 33}$ = 2.279; P < 0.05), although we did not find any significant differences between males and females ($t_{1, 19}$ = -1.615; P = 0.123). Mass-specific SMR increased with an increase in in T_a ($t_{1, 33}$ = 13.364; P < 0.0001) but decreased with an increase in M_b ($t_{1, 33}$ = -6.327; P < 0.001). There were no significant differences in mass-specific SMR between males and females ($t_{1, 19}$ = 0.414; P = 0.687).

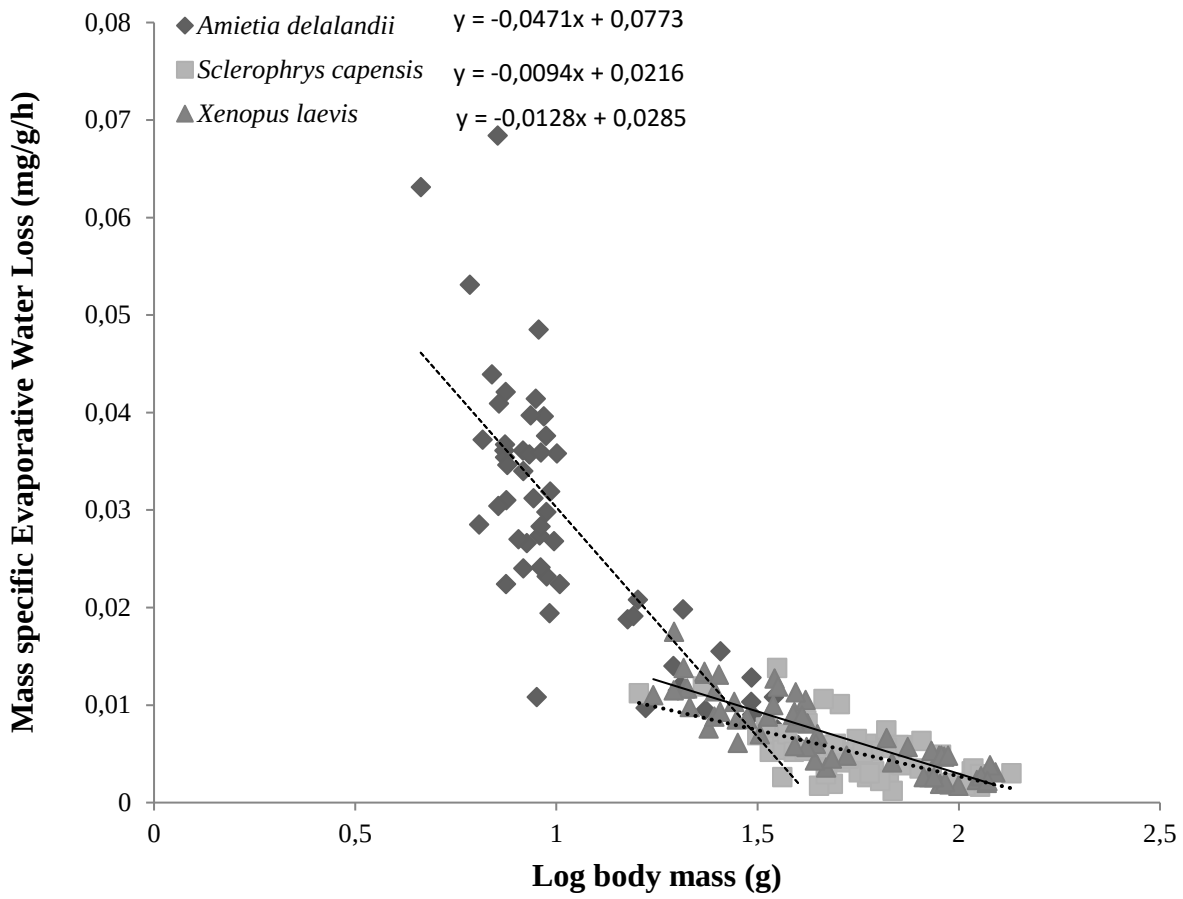

Figure 4.4. Mass-specific evaporative water loss of different functional groups as a function of body mass (M_b) at all experimental temperatures.

Interspecific variation

Species difference in whole-animal EWL and SMR are confounded by differences in M_b. Here I limit the interspecific differences to the two species with comparable body sizes: *X. laevis* and *S. capensis*. I found significant differences in T_b between the two species only at T_a = 5°, 25° and 35°C. At T_a = 5°C, *X. laevis* had significantly higher T_b ($t_{3, 12}$ = 3.427; P < 0.01) and that T_b increased with an increase in

M_b ($t_{1, 12}$ = 3.425; P < 0.01). At T_a = 25°C, *S. capensis* had significantly higher T_b ($t_{1,12}$ = 2.225; P < 0.05) and T_b increased with an increase in M_b ($t_{1, 12}$ = -3.425; P < 0.01). At T_a = 35°C, *X. laevis* had significantly high T_b compared to the *S. capensis* ($t_{1,12}$ = 4.148; P < 0.0001) and I found that M_b did not have a significant effect on this relationship.

I observed differences in whole-animal EWL at T_a between 10° and 25°C (T_a = 10°: $t_{(1,12)}$ = -2.999; P < 0.05, T_a =15°: $t_{(1,12)}$ = -4.598; P < 0.001, T_a =20°: $t_{(1,12)}$ = -4.598; P < 0.001, T_a =25°: $t_{(1,12)}$ = 6.027; P < 0.0001). *Sclerophrys capensis* had significantly higher rates of whole-animal EWL at T_a = 10° and 20°C and *X. laevis* had significantly higher rates of whole-animal EWL at T_a = 15° and 25°C. I also found significant differences in rates of SMR at T_a between 15° and 25°C (T_a = 15°: $t_{(1,13)}$ = 3.987; P < 0.001, T_a = 20°: $t_{(1,11)}$ = -2.439; P < 0.05, T_a = 25°: $t_{(1,12)}$ = 5.328; P < 0.001). *Xenopus laevis* had significantly higher rates of SMR at T_a = 15° and 25°C and *S. capensis* at T_a = 20°C. Lastly, M_b only influenced this relationship at T_a = 35°C for EWL ($t_{(1,14)}$ = 3.356; P < 0.001) and SMR ($t_{(1,14)}$ = 3.546: P < 0.05) such that both EWL and SMR increased with an increase in M_b.

Discussion

One of the greatest challenges for life in amphibians is that both metabolism and thermoregulation are not only coupled, but also controlled by the same external factors such as environmental temperature. Thus, this study aimed to assess how the variation in T_a affects key physiological traits in three different African anuran species with different ecologies. I found that T_a had a significant positive effect on T_b, whole-animal EWL, whole-animal SMR, mass-specific EWL and mass-specific SMR for all species. Secondly, M_b influenced T_b, in *A. delalandii* and *S. capensis*, but not in *X. laevis*. Furthermore, M_b did not influence rates of whole-animal EWL in all three species, but I found that whole-animal SMR increased with an increase in M_b only for *S. capensis*. Body mass negatively influences both rates of mass-specific EWL and SMR for all species. Lastly, I did not find any difference between the sexes in T_b, EWL, SMR, both mass-specific EWL and SMR for all three species.

Body temperature

The concept of thermal inertia suggests that although larger individuals take longer to warm up, they also take longer to cool down (Carey, 1978). A study looking at how M_b affects T_b in different toad species concluded that larger individuals had higher thermal inertia than small sized individuals (Carey 1978). In addition, Newman & Dunham (1994) found that *Scaphiopus couchii* toadlets that metamorphosed at larger sizes took longer to reach critical dehydration levels compared to small-sized toadlets. Collectively, these results highlight the importance of M_b as a significant factor influencing

key physiological processes such as T_b through its effect on rates of heating and cooling (see Wygoda 1988a) particularly in vertebrate ectotherms. In this study, I found that T_b increased with an increase in M_b in *A. delalandii* and *S. capensis*. These results suggest that the effect of M_b on heat flux may be more beneficial for species spending a large proportion of their time on land, where changes in temperature are more pronounced and may be sudden.

I observed species specific differences in T_b at T_a = 5°, 25° and 35°C and T_b increased with M_b at these test temperatures. This result seems to suggest that although M_b is a key factor affecting T_b in amphibians, this may not be the case across a broad range of available environmental temperatures and species ecologies. Furthermore, I found no significant relationship between M_b and T_b in *X. laevis* despite the species showing the largest difference in sexual dimorphism (see Table 4.1). Wygoda (1988a) suggested that prolonged cooling may be adaptive as it assists anurans to maintain a higher T_b that is essential for performance under decreasing temperature conditions. Hence amphibians are expected to have larger body sizes in more temperate, cooler environments (Ashton 2002), although the generality of this accretion has been challenged, particularly in largely aquatic urodeles (Olalla-Tárraga & Rodríguez 2007). It may also be interesting to see the type of pattern that emerges if anurans would be differentiated by functional groups.

Evaporative water loss

I found that whole-animal EWL increased with an increase in T_a for all our species. I expected this result because amphibians use evaporative water loss to counter exposure to higher temperatures and water loss increases with an increase in metabolic rates. I also wanted to determine whether different species differ in their ability to regulate water loss (e.g., Wygoda, 1984; Young et al., 2005). Both Wygoda (1984) and Young et al. (2005) concluded that species that adopted an arboreal lifestyle show significantly reduced level of water loss. In addition to arboreal lifestyle, (Tracy et al. 2010) concluded that both high rates of cutaneous resistance to water loss (R_c: the ability to reduce rates of water loss), and larger M_b were important in reducing desiccation time in amphibians. However, in this study I did not find any significant effect of M_b and sex on rates of whole-animal EWL in the three-species considered. Furthermore, there seems to be a difference in the onset of evaporative cooling with high R_c/atypical frog species only employing EWL at high ambient temperatures compared low and moderate R_c/typical frogs (Tracy et al. 2008). Species with high rates of R_c are reported to have reduced EWL and have been observed to increase their T_b above ambient as an adaptation to terrestrial habitats (Buttemer 1990; Tracy & Christian 2005).

I expected that *S. capensis* would be capable of maintaining T_b close to T_a, better than the other two species and perhaps delay the onset of EWL mechanisms to cool down. I found that T_b was lower than

T_a for all our species, particularly at high T_a suggesting that all species were using evaporative cooling at high T_a. But at T_a between 25° and 35°C, where T_b slightly deviates from T_a (see Figure 4.1), *S. capensis* had significantly higher T_b at T_a = 25° and 30°C, perhaps suggesting the delayed onset of evaporative cooling in this species (see also Lillywhite 1971). This largely follows the results of Young et al. (2005) who concluded that although the invasive cane toad (*Rhinella marina*) had moderate rates of R_c, it was still 'atypical' as compared to 'typical', mainly semi-aquatic and stream-breeding frogs. In arboreal species, Tracy et al. (2008) found that species with low to moderate rates of R_c increase EWL at higher ambient temperatures to regulate T_b. Indeed, while studying toads, Tracy (1978) postulated that toads possess an ability to withstand higher T_b for longer periods, provided that the skin remains moist. Although the ability to regulate water loss is important for amphibians (e.g. tree frogs), other factors such as the ability to rehydrate quickly and absorb water from a variety of substrates (e.g. burrowing species) may have been as important for amphibians to invade such a variety of terrestrial habitats through the course of evolution (Cartledge et al. 2006; Prates & Navas 2009).

Standard metabolic rates

Whole-animal SMR increases with an increase in T_a as a result of the increase in kinetic energy and reaction rate required at high temperatures (Brown et al. 2004; Clarke 2006; Gillooly et al. 2001). I expected *S. capensis* to have comparatively low rates of SMR (particularly at high T_a), as an adaptation to terrestrial life because: i) food and water becomes limiting in terrestrial habitats and terrestrial specialists should adopt a sit-and weight foraging strategy, although see Pough & Tiagen (1990), or ii) only be active nocturnally or at low to intermediate levels of T_a to reduce rates of EWL (Peterman & Semlitsch, 2014). Indeed, I found that *S. capensis* had significantly lower rates of metabolism at both 15°, 25° and 35° C. Furthermore, whole-animal SMR increased with an increase in M_b only in *S. capensis*. During species comparisons, I also found that M_b increased with an increase in both whole-animal EWL and SMR only at T_a = 35° C. This result is particularly interesting in that it may suggests that in Africa's temperate south, where most species are not experiencing freezing conditions, as it is the case in the northern hemisphere, large bodies confer an advantage in delaying warming rates.

Ecological specialisations usually occur as a direct result of adaptation to a finite set of encountered environments (Poisot et al. 2011). My results suggest that although variation in T_a is important in determining T_b, EWL and SMR in amphibians, not all amphibians are affected in a similar fashion. Although M_b and sex (given the pronounced sexual dimorphism in anurans) have been identified as key factors affecting physiological traits in anurans, I suggest that perhaps this result should be considered in the context of each species' prevailing ecology. However, certain caveats to this assertion need to be considered. First, I do acknowledge that the observed differences could have been because the species that we considered represent very divergent groups (three different anuran families) so that the

differences are a function of the divergent evolutionary history as opposed to different ecologies (but see Tracy & Christian, 2005). Nevertheless, Young et al. (2005) and Tracy et al. (2008) found that ecologically mediated differences in water loss rates were observable even within clades and further concluded that resistance to water loss might have evolved multiple times within hylids. These results further highlight the importance of ecology.

In conclusion, I found that T_a has a significant influence on key physiological traits in all functional groups investigated. I found that M_b only influenced T_b and whole-animal SMR in *S. capensis* and only T_b in *A. delalandii*. Furthermore, M_b was found not to impact rates of whole-animal EWL, irrespective of species ecology. These results suggest that the importance of M_b in influencing key physiological factors should be looked at in the context of species ecology (e.g., Olalla-Tárraga & Rodriguez, 2007). This is particularly important in anurans where some species may respond changes in climate by altering their body sizes (e.g., Sheridan et al. 2018). Despite the pronounced sexual dimorphism in anurans, sex did not influence the relationship of T_a with all physiological variables considered. I suggest future studies should focus on disentangling the importance of M_b in phylogenetically related, non-arboreal, African anurans as information of how T_a affects key physiological traits in these species is currently lacking. Furthermore, future work should be undertaken to understand energy demands of different species, such as stream breeding species, given the threats that they currently face (Sodhi et al. 2008) particularly when considering the expected effects of climate change on amphibians (Buckley et al. 2012; Deutsch et al. 2008).

Chapter five: Effets of temperature on locomotor performance trait relationships (burst speed and ensurance) in southern Africa frogs

Abstract

Temperature regulates most, if not all important physiological processes in ectotherms. To minimise the metabolic costs of performance, ectotherms have evolved to perform physiological processes such as locomotor performance at varying environmental temperatures. The aim of this study was to determine if: i) variation in ambient temperature (T_a) affects burst swimming and hopping (velocity and acceleration) and endurance (distance and time) in different African frog species, ii) to determine whether different species respond differently to changes in temperature (thermal reaction norms) and iii) whether temperature affects how species consistently outperform each other based on life history specialisations. Experiments were conducted at five different test temperatures (10°-30° C, at 5° C intervals), on three temperate African anuran species with different ecologies: i) the principally aquatic African clawed frog (*Xenopus laevis*), ii) the stream breeding common river frog (*Amietia delalandii*) and iii) the largely terrestrial raucous toad (*Sclerophrys capensis*) based on their apparent dependency on water. I found that T_a had a significant impact on all performance traits. Furthermore, there were significant differences in species reaction norms only in terrestrial performance traits. *A. delalandii* outperformed *X. laevis* in both speed and acceleration traits in burst swimming and as well as hopping experiments, respectively – despite *X. laevis* being adapted to an aquatic lifestyle. *S. capensis* outperformed *X. laevis* and *A. delalandii* in hopping endurance trials (distance and time), despite changes in T_a. Lastly, the extent to which the variation in temperature affects terrestrial performance traits, may suggest that given the predicted changes in climate, anurans with a terrestrial life stage may experience performance related challenges or be forced to perform at environmental temperatures close to or beyond optimum, leading to reduced thermal breadths, activity periods and subsequently, reduced distribution ranges.

Introduction

The balance between an animal's ability to assimilate energy essential for growth and reproduction, including the need to dissipate excess energy, determines their fitness (Angilletta et al. 2002; Arendt 2009; Navas et al. 2008; Seebacher & Franklin 2011; Townsend & Stewart 1986). For animals that lack the ability to regulate their body temperature (T_b) internally such as ectotherms, these fitness-related physiological processes take place at varying temperatures (Huey & Stevenson 1979), compared to endotherms that maintain a constant T_b. It has already been shown in both lizards and frogs that the temperatures selected while at rest in laboratory thermal gradient experiments are usually higher than temperatures where they are found in the wild (Huey 1991), prompting suggestions that environmental temperatures may set a limit to how ectotherms can efficiently perform key physiological functions in the wild (Hillman et al. 2009). This is compensated by both broad performance breadths evident in most ectotherms (John-Alder et al. 1988), particularly amphibians. Most anurans are thermo-conformers with exceptional levels of plasticity in performance measures, as a function of temperature (see Tingley et al. 2012; Urban et al. 2014; Wilson & Franklin 1999; Wilson et al. 2000).

Several studies have looked at the relationship between the temperature selected by different ectotherm species in the laboratory and how this relates to optimum performance temperature (T_{opt}: Bartholomew 1958; Huey & Stevenson 1979). However, most of these studies have also highlighted that T_{opt} is influenced by other physiological variables such as nutritional state and more specific to amphibians; hydration levels (Preest & Pough 1989; Preest & Pough 2003). In addition, T_{opt} only provides a snapshot of physiologically relevant temperatures, and may thus mask some performance-temperature relationships that may not be temperature sensitive at one specific temperature (John-Alder et al. 1988). Furthermore, T_{opt} seems to vary between different performance traits (Herrel & Bonneaud 2012b; Preest & Pough 2003; Titon Jr et al. 2010; Wilson et al. 2000), sometimes leading to trade-offs.

How species outperform one another in differebt performance traits driven largely by the interplay between morphology and performance (see Toro et al. 2004; Vanhooydonck et al. 2001). Indeed, one of the best studied whole-animal performance trade-off systems is the trade-off between burst speed and endurance locomotor performance traits (Herrel & Bonneaud 2012b; Vanhooydonck et al. 2014; Vanhooydonck et al. 2001; Wilson et al. 2002). There is mounting evidence supporting the view that species inhabiting similar habitats, show similar morphological adaptations and performance, irrespective of phylogeny (Moen et al. 2013). Among anurans, species inhabiting aquatic environments are presumed to perform better at burst swimming velocity as opposed to endurance (John-Alder et al. 1989; Putnam & Bennett 1981). This pattern has also been observed within species, where individuals that perform better at burst performance show reduced endurance capacity (Careau et al. 2014; Herrel & Bonneaud 2012b; Herrel et al. 2014; Wilson et al. 2002).

Anurans are unique among land vertebrates in that most display a complex bi-phasic life style. Some completely bypass the aquatic larval stage (direct development) while others spend most of their lives in aquatic environments leading to the high variation in life-history traits. These different ecologies are largely based on the site of egg deposition including tadpoles development (Mercurio et al. 2009; Wells 2007) and a range of adult morphological attributes (Bossuyt & Milinkovitch 2000; Hillman et al. 2009; Moen et al. 2013). This high variation in life-histories suggests that anurans will exhibit an equally remarkable variation in how they respond to environmental challenges as mediated by differences in habitats and this will be reflected in how they perform specific tasks.

The aim of this study is first to determine the effect of temperature on burst swimming and hopping (velocity and acceleration) and hopping endurance (distance and time) in different frog species. To achieve this, I chose three different, widely distributed, temperate African anuran species with different ecologies based on their apparent dependency on water: i) the principally aquatic African clawed frog (*Xenopus laevis*), ii) the stream breeding, semi-aquatic common river frog (*Amietia delalandii*), and iii) the largely terrestrial raucous toad (*Sclerophrys capensis*). Secondly, I aim to determine whether different species respond differently to changes in temperature (thermal reaction norms). And lastly, I want to determine if temperature affects how species outperform each other as a function of their ecologies. I expect the principally-aquatic species to perform best at maximum burst swimming performance. The semi-aquatic species is expected to do well at maximum terrestrial hopping acceleration (quickness of movement). Lastly, I expect the terrestrial species to show maximum terrestrial endurance capacity (see Putnam and Bennett 1981). These species were chosen because in addition to inherent differences in habitat specialisation, they are sympatric and occupy the same climatic niche (chapter three). Moreover, they also show differences in locomotor specialisations and behaviour (Mendiola et al. 1991; Taigen & Pough 1983).

Methods

Animals

Adult individuals of the three species were caught around the area of Port Elizabeth, South Africa (see Table 4.1) excluding gravid females as suggested by Hillman et al. (2009). I used the search and seize method for both *A. delalandii* and *S. capensis*. *Xenopus laevis* was caught using bucket traps placed in ponds baited with chicken livers. Upon capture, I recorded body mass of each individual as well as the GPS coordinates for each site of capture. I caught between 20 and 30 individuals to reduce the chances of over-exertion of individuals during experiments (see below). Animals were kept for a maximum period of 2-4 months in the lab using environmentally enriched conditions, until all experiments were completed. After completing the experiments, animals were returned to their respective sites of capture.

Amietia delalandii were maintained in 110 L terrariums, with sand and small logs for cover, at low densities (5 individuals per box). *Sclerophrys capensis* were kept in 'terraria' made from circular paddling pools (*d* × *h*: 2.16m × 0.45m - ≈ 1 600 L capacity) with sand, water and small logs and bark to provide cover. Both species were fed mealworms and crickets, dusted with calcium and other mineral supplements (ReptiCalcium, Zoo Med Laboratories Inc; South Africa). *Xenopus laevis* were kept in a fresh water tank (*l* × *b* × *h*: 3.55m × 0.9m × 0.63m) with stacked-bricks and stones to provide cover and were fed a diet of ox heart. All species were fed *ad libitum*, 3 times a week and were kept in temperature-controlled holding rooms on a 12:12 (light: dark) photoperiod set at 20° C by circulating atmospheric air to maintain a constant temperature into the holding rooms. Ambient humidity levels fluctuated with outside air. Water was provided throughout the duration of the study (including periods when food was withheld) to prevent dehydration stress. I further ensured that the 'terraria' in which *A. delalandii* and *S. capensis* were held; was sprayed with water every 3 days to dampen the sand and provided a bowl with water daily where anurans could rehydrate *ad libitum*. To identify each individual, I branded *X. laevis* and *A. delalandii* with unique three digit number brands, using liquid nitrogen (see Measey & Tinsley 1998, where a similar branding method was followed). The unique individual patterning on the dorsal side of *S. capensis* was used to differentiate between different individuals.

Morphological data

In addition to body mass (see Table 4.1), other morphological measurements taken prior to performance experiments, using digital (0.01 mm) Vernier callipers (Grip® Lutron Electronic, Johannesburg) were: Snout-Vent Length (SVL), Snout-Urostlye Length (SUL), Head Length (HL), Head Width (HW), Head Height (HH), Femur Length (FL), Tibia length (TB), Metatarsal (MT), Foot Length (FL), Longest Toe (LT), Humerus length (HM), Radius length (RD), Metacarpal (MC), Longest finger (LF), following a detailed morphological procedure by Herrel et al. (2012).

Performance

Burst swimming and hopping speed

Burst speed performance traits (burst swimming and burst hopping) were measured by randomly selecting 10 individuals (5 males and 5 females), while ensuring minimal over-exertion on individuals (rotating individuals between different temperature trials). Experiments were conducted in a temperature controlled room, at a range of environmental temperatures, from 10° to 30° C at 5° C intervals (Herrel & Bonneaud 2012a; Herrel & Bonneaud 2012b; Wilson et al. 2000). Target T_a in the climate rooms was set overnight before commencing with experiments and I proceeded only when the temperature was within 1° C of the targeted temperature. This was achieved by reconciling the

temperature on the mercury thermometer in the climate room with the temperature recorded using a thermocouple probe (Fluke 54IIB, Fluke Corporation, Washington). Test temperatures were also selected at random to minimise any effect of a directional change in temperature on performance traits. Prior to experimentation, animals were placed for one hour in individual containers with water in an incubator set at the target temperature (Navas 1996). All performance measurements were repeated twice for each individual with an inter-trial period of approximately one hour, during which animals were returned to the incubator and allowed to recover. Mass was recorded to the nearest ±0.01 g (Adam Equipment Portable Balance; 300G 230V, South Africa) before the first experimental run. I also ensured that body temperature (T_b) was within 1° C of the target T_a for each trial by recording the cloacal temperature using a thermocouple probe (Fluke 54IIB, Fluke Corporation, Washington) before and after each trial.

Burst swimming and hopping speed trials were conducted in a clear Perspex tank ($l \times b \times h$: 2m × 0.4m × 0.4m), marked at 25 cm intervals (at the base and on the sides). I mounted a mirror ($l \times b$: 1.4m × 0.4m) above the tank, at a 45° angle, to ensure that during trials, animals were visible both from the dorsal and lateral views. Only the best view was used during analysis (see below). Burst of speed performance was recorded using a high speed digital video camera (Canon *PowerShot* G15, Canon Inc., Japan) set at 240 frames per second (*fps*). Swimming and hopping burst speed was initiated by a light tap on the urostyle. Only video clips of good contrast and where the frog swam continuously and in a straight line for two or more consecutive strokes were retained. The video clips were then processed and cropped to include at least two locomotor cycles and analysed manually. For each frame, the snout tip was digitized and tracked across all cropped frames that successfully captured the identified two locomotor cycles, using an image tracking software Blender (version 2.74; Blender Foundation, Amsterdam). The coordinates of the animals' movement were exported to Excel (Microsoft, Redmond, WA, USA) and the displacement of the individual along its path was calculated. The raw displacement profile was then smoothed by using a zero phase shift low-pass Butterworth filter version 2 with the cut-off frequency set at 30Hz adapted from (Winter 2005). The final maximum velocity and acceleration were calculated by numerical differentiation of the smoothed displacement profiles. For each individual, I extracted the highest instantaneous velocity and acceleration across all swimming/hopping sequences recorded: i.e. the fastest velocity and acceleration may thus come from different sprint swimming/hopping performance trials (Herrel & Bonneaud 2012a; Herrel & Bonneaud 2012b; Herrel et al. 2012; Herrel et al. 2014; Miller 1982). The base of the Perspex tank was covered with cork tiles to improve traction during these burst hopping experiments.

Endurance capacity

For endurance experiments, six individuals of each species (3 males and 3 females) were randomly selected. I chased an individual animal along a 4.7 m circular track, made from a circular paddling pool ($d \times h$: 2.16m × 0.45m ≈ 1 600 L capacity) until exhaustion. This was expressed as the point at which the individual was not able to right itself when placed on their backs (see Herrel & Bonneaud 2012a; Herrel & Bonneaud 2012b). Endurance experiments were only undertaken on a dry surface (maximal hopping endurance). Mass was recorded to the nearest ±0.01 g before and after the experimental run. I also ensured that T_b was within 1° C of the target T_a before trial by recording the cloacal temperature using a thermocouple probe (Fluke 54IIB, Fluke Corporation, Washington). The floor of the track was covered with a high friction matting (GECKO® Non-slip matting, Cape Town) to improve traction. For each individual, I recorded both the total distance covered and the time spent moving until exhaustion (Herrel & Bonneaud 2012a).

Statistical analysis

Evidence suggests that performance in anurans is size dependent (see Bennett et al. 1989; Nauwelaerts et al. 2007). Thus, all performance measures were size corrected before conducting any analysis. I performed normality tests on the predictor variables for each individual species separately. This is because I first wanted to determine if different performance traits are temperature sensitive for each distinct species, as suggested by Angilletta (2006). Subsequently, I used log transformation to normalise data, where necessary. I then used both parametric (t-test) and non-parametric (Wilcox test) statistics, where necessary to determine if there were significant differences between pairs of species, where performance trade-offs were most likely (Supplementary figure S5.1). These tests were conducted over a range of study temperatures (see above) to determine if T_a influences the relationship between performance trade-off among different performance traits. All statistical tests were carried out in R (R Development Core Team 2017).

Results

Burst swimming speed

There were significant differences in burst swimming velocity among species at all test temperatures: T_a = 10° C ($F_{2, 26}$ = 11.49; P < 0.0001), T_a = 15° C ($F_{2, 27}$ = 13.49; P < 0.0001), T_a = 20° C ($F_{2, 25}$ = 9.56; P < 0.0001), T_a = 25° C ($F_{2, 25}$ = 8.013; P < 0.0001) and T_a = 30° C ($F_{2, 26}$ = 20.68; P < 0.0001; see Figure 5.1). Following a TukeyHSD test, I found that *S. capensis* had significantly lower burst swimming velocity compared to both *X. laevis* and *A. delalandii*. I found that *A. delalandii* consistently outperformed *X. laevis* burst swimming velocity which was contrary to the predictions (see Supplementary figure S5.1), *A. delalandii* had higher burst swimming velocity that *X. laevis* at all test

temperatures (t = 6.708; $P < 0.001$), except at T_a = 20 ° C (t = 1.482; $P = 0.155$) where there were no significant differences between the two species. I did not find a significant interaction term between species and temperature ($\chi^2 = 14.089$; $P = 0.079$) suggesting that temperature had a similar effect on burst swimming velocity among different species.

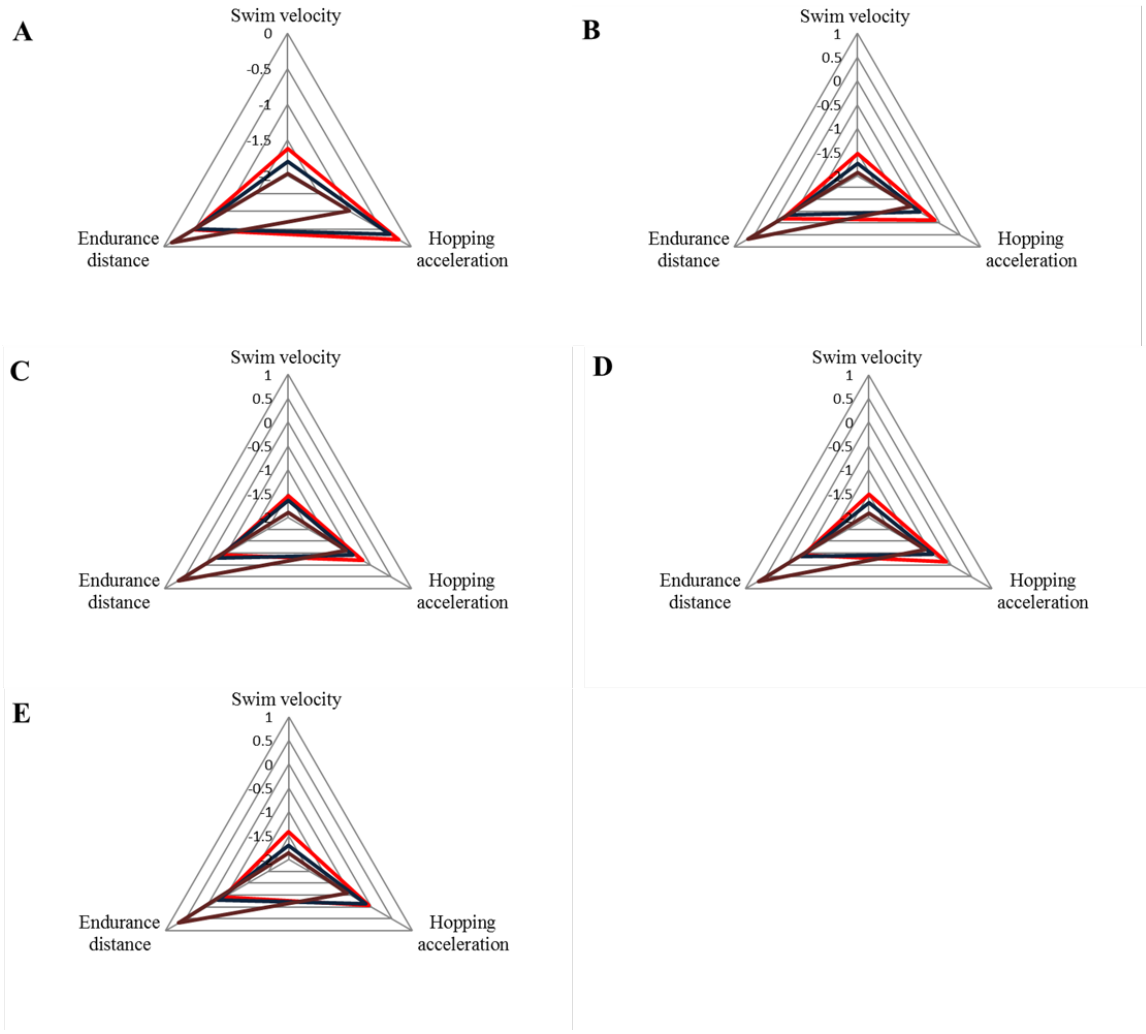

Figure 5.1. Spider plot showing log-transformed, size-corrected performance relationships between different performance traits and species with different ecologies. The relationships were assessed at different temperatures (A-E): A = 10°, B = 15°, C= 20°, D = 25° and E = 30° C. Different coloured lines denotes different species: Red = *Amietia delalandii*; Brown = *Sclerophrys capensis* and dark blue = *Xenopus laevis*.

For aquatic acceleration, I also found significant differences among species at all test temperatures: T_a = 10°C ($F_{2, 26} = 10.32$; $P < 0.0001$), T_a = 15°C ($F_{2, 27} = 24.48$; $P < 0.0001$), T_a = 20° C ($F_{2, 25} = 7.417$; $P < 0.0001$), T_a = 25°C ($F_{2, 25} = 13.63$; $P < 0.0001$) and T_a = 30°C ($F_{2, 26} = 22.02$; $P < 0.0001$). These results were augmented with a TukeyHSD test which suggested that *S. capensis* accelerated significantly more slowly compared to *X. laevis* and *A. delalandii* at all test temperatures. I did not find significant

differences in swimming acceleration between *X. laevis* and *A. delalandii*, except at T_a = 30° C, where *A. delalandii* accelerated faster than *X. laevis*. Temperature had a similar effect on burst swimming acceleration across the different species (χ^2 = 8.459; P = 0.389).

Burst hopping speed

I found that temperature affected each species differently, leading to different temperature reaction norms for both burst hopping velocity (χ^2 = 33.488; P < 0.001) and acceleration (χ^2 = 45.211; P < 0.001). Furthermore, significant differences were also found in burst hopping velocity and acceleration among different species at all test temperatures (see Figure 5.1). At T_a = 10° C (velocity: $F_{2.26}$ = 24.07; P < 0.0001, acceleration: $F_{2.26}$ = 27.11; P < 0.0001) and T_a = 20° (velocity: $F_{2.26}$ = 11.93; P < 0.0001, acceleration: $F_{2.26}$ = 12.82; P < 0.0001), *S. capensis* had significantly lower burst hopping velocity and acceleration when compared to *X. laevis* and *A. delalandii*, following a TukeyHDS test. Nevertheless, there were no significant differences in both velocity and acceleration between *A. delalandii* and *X. laevis* at T_a = 10° and 20° C. At T_a = 15° C, I found significant difference in burst hopping acceleration among different species, whereby *A. delalandii* had significantly higher burst hopping velocity, followed by *X. laevis* and lastly *S. capensis*. *Sclerophrys capensis* had significantly slow hopping acceleration compared to *X. laevis* and *A. delalandii* at the remaining test temperatures, although there were no significant differences between *A. delalandii* and *X. laevis* (see figure 5.1). I found evidence that *A. delalandii* accelerated significantly faster when compared to *S. capensis* at all test temperatures (t = 15.744; P < 0.0001) while *S. capensis* had the highest hopping endurance at all test temperatures (see Supplementary figure S5.1).

Hopping endurance

I found a significant interaction term between species and temperature, suggesting that temperature did not have a similar effect on distance travelled until maximum exertion among different species (χ^2 = 21.593; P < 0.001). There were significant differences in the distance travelled until maximum exhaustion (endurance capacity) such that *S. capensis* travelled significantly farther before reaching maximum exertion at all test temperatures: T_a = 10° C ($F_{2, 14}$ = 49.47; P < 0.0001), T_a = 15° C ($F_{2, 15}$ = 44.85; P < 0.0001), T_a = 20° C ($F_{2, 15}$ = 21.29 ; P < 0.0001), T_a = 25° C ($F_{2, 15}$ = 34.08; P < 0.0001), and T_a = 30° C ($F_{2, 14}$ = 49.47; P < 0.0001 see figure 5.2). I subsequently ran a TukeyHSD test to determine where the differences in distance travelled until exhaustion and found that at T_a = 10° C, *S. capensis* travelled significantly farther than *X. laevis*, which also travelled farther when compared to *A. delalandii*. I did not find any significant differences between *X. laevis* and *A. delalandii* at the remaining

temperatures. This result suggests that *S. capensis* had significantly higher hopping endurance distance performance capacity than *X. laevis* at all test temperatures (W = 700; $P < 0.0001$).

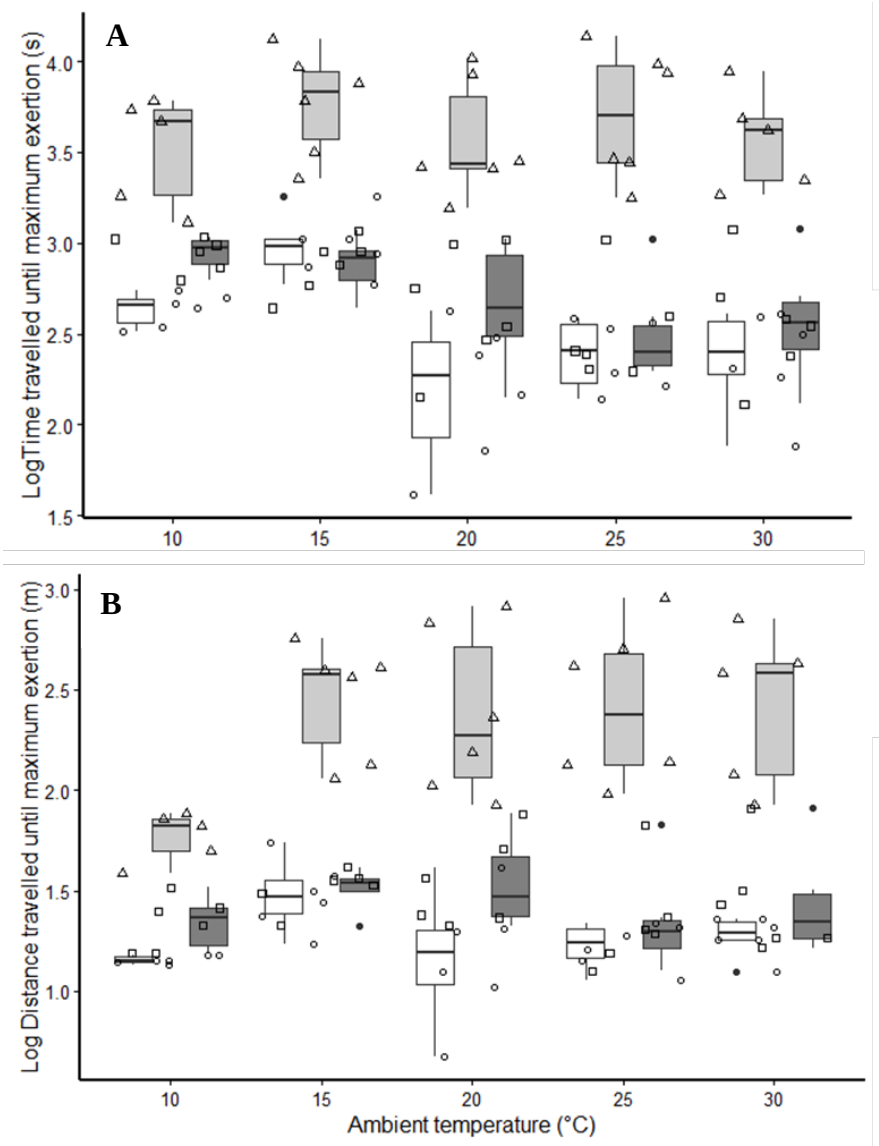

Figure 5.2. Log-transformed endurance performance traits recorded at different ambient temperatures. A: time travelled until exhaustion, B: distance travelled until exhaustion for different species. Circles = *Amietia delalandii*; triangles = *Sclerophrys capensis* and squares = *Xenopus laevis*. Boxplots: open = *Amietia delalandii*; light grey = *Sclerophrys capensis* and dark grey = *Xenopus laevis.*

Lastly, I found that temperature did not have the same effect on the distance travelled to maximum exertion in the three species ($\chi^2 = 22.152$; $P < 0.001$), thus leading to different reaction norms. There were significant differences in the time it took for different species to reach maximum exertion. The differences were largely between *S. capensis* and the other two species at all test temperatures: $T_a = 10°$ C ($F_{2, 14} = 33.02$; $P < 0.0001$), $T_a = 15°$ C ($F_{2, 15} = 31.55$; $P < 0.0001$), $T_a = 20°$ C ($F_{2, 15} = 24.17$; $P <$

0.0001), T_a = 25° C ($F_{2, 15}$ = 39.03; P < 0.0001) and T_a = 30° C ($F_{2, 14}$ = 26.18; P < 0.0001; see Figure 4.2). Subsequently, in a TukeyHSD test, I found that at 10° C, there were significant differences between all 3 species, with *S. capensis* taking longer to reach maximum exertion, followed by *X. laevis* and lastly *A. delalandii*. *Sclerophrys capensis* took longer to reach maximum exertion at 15° to 30° C test temperatures. I could not detect any significant differences between *X. laevis* and *A. delalandii* at T_a = 15° to 30° C.

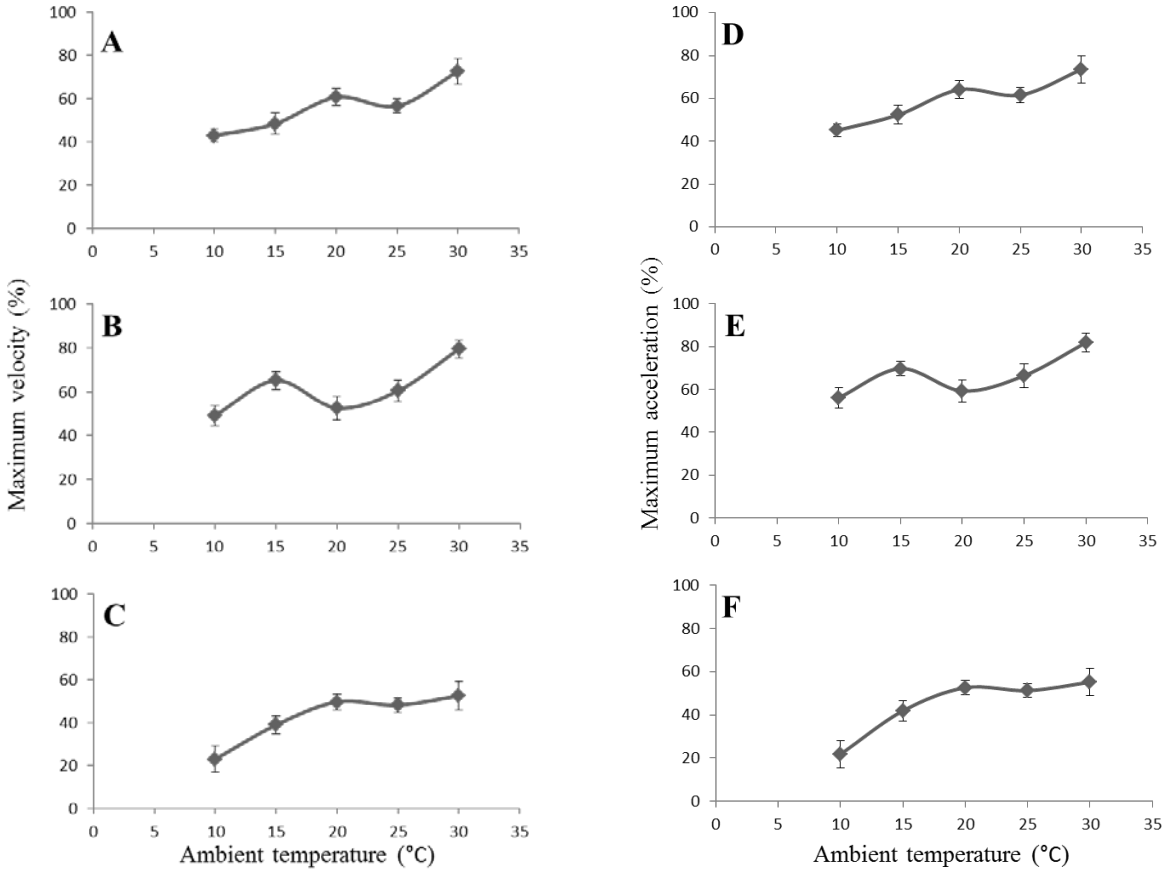

Figure 5.3. Relative maximum burst hopping performance traits recorded at different ambient temperatures (speed and acceleration) for different species: A and D: *Xenopus laevis*, B and E: *Amietia delalandii* and C and F: *Sclerophrys capensis.*

Discussion

Although the importance of temperature in determining performance in ectotherms may not be disputed, this study suggests that the extent to which temperature affects performance varies between traits and mediums where experiments are conducted. I further observed that the extent to which T_a affects performance was not similar between species (see Angilletta et al. 2003; Richter-Boix et al. 2015). I

found that there was a significant interaction term between T_a and species particularly for terrestrial burst hopping (velocity and acceleration) and endurance capacity (time and distance). Most importantly, thermoregulation and evaporative cooling are intricately linked in anurans (Beuchat et al. 1984), perhaps influencing the temperature-performance relationship. However, I did not find a significant interaction term between performance and species (reaction norms) in burst swimming performance traits as predicted (velocity and acceleration), highlighting the importance of the inherent need to thermoregulate in each of the media (aquatic *vs* terrestrial environments) in a group where performance is also linked to hydric state and water loss (Hillman et al. 2014; Titon Jr et al. 2010).

The differences in performance-temperature reaction norms between different species could be further attributed to the observation that performance plateaued at mid to high temperatures; in *S. capensis* for burst hopping (velocity and acceleration, figure 5.3) and burst swimming (acceleration and distance see figure 5.4), while it increased at low temperatures and plateaued at high temperature for endurance traits in both *A. delalandii* and *X. laevis*. This result could provide support to the suggestion that the lack of a relationship between performance and temperature could be driven by the performance trait *vs*. fitness trade-off (see Angilletta et al. 2002). This trade-off suggests that a species will show reduced thermal sensitivity in a performance trait that does not contribute positively to its fitness (Angilletta et al. 2003; Gilchrist 1995; Huey & Kingsolver 1993). The existence of such a trade-off could be driven by other factors such as resource acquisition and anti-predatory behaviour. Putnam and Bennett (1981) found that *Anaxyrus boreas* had higher sustained performance over a longer period and thus covered longer distances compared to *Lithobates pipiens*. Moreover, the short bout sustained hops characteristic of toads is maintained by aerobic respiration, while rapid jumps by ranid species are largely sustained by anaerobic catabolism (Bennett & Licht 1973; Seymour 1973, see also Reilly et al. 2015).

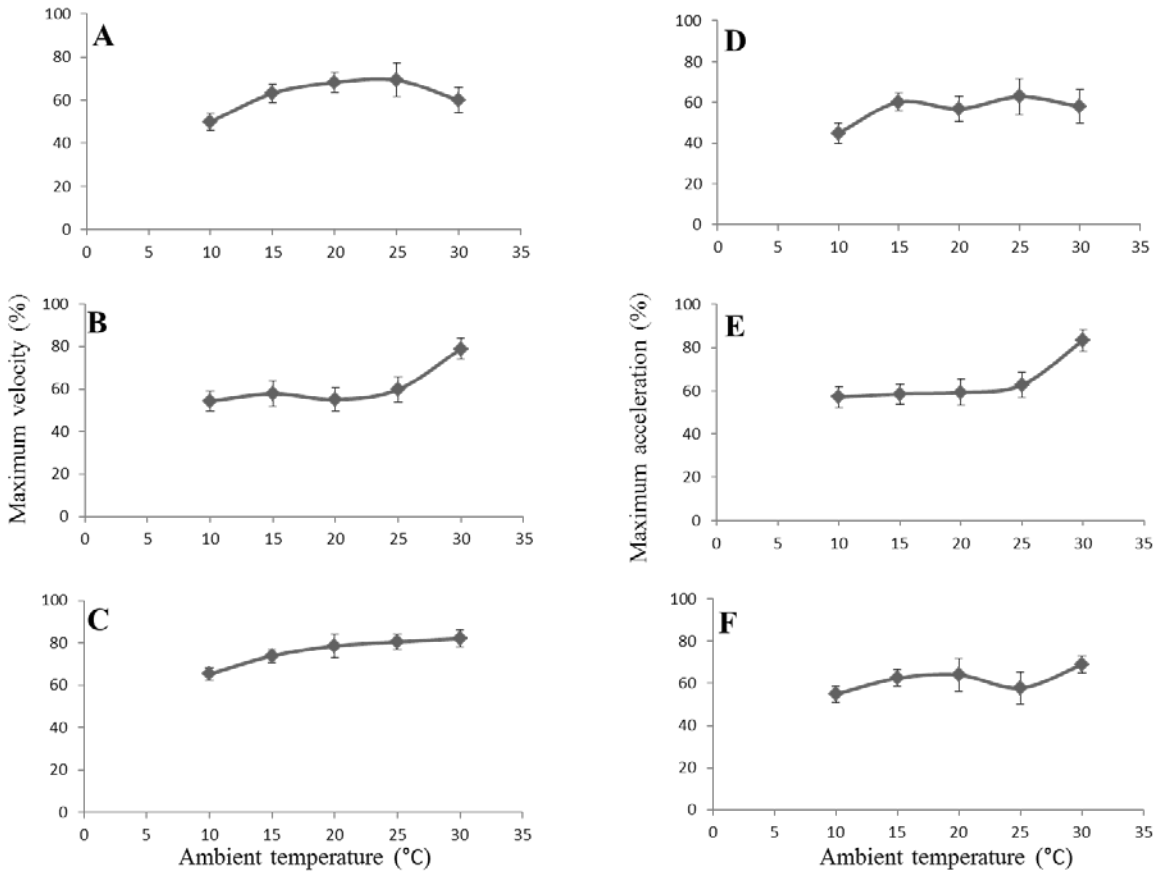

Figure 5.4. Relative maximum burst swimming performance traits recorded at different ambient temperature (speed and acceleration) for different species. A and D: *Xenopus laevis*, B and E: *Amietia delalandii* and C and F: *Sclerophrys capensis.*

This ability of toads to sustain movement for longer periods and over larger distances and its bounded, energy efficient nature (Reilly et al. 2015) is described as one of the crucial factors explaining the bufonids' global footprint (e.g., van Bocxlaer et al. 2010), as well as the successful invasion of cane toads (*Rhinella marina*) into warmer parts of Australia (see Seebacher and Franklin, 2011). Furthermore, Llewelyn et al. (2010) found that populations that are at the front of an invasion had greater endurance compared to core populations in cane toads, although recent evidence suggest that this may be a behaviourally plastic trait (Pettit et al. 2016). On the other hand, both *A. delalandii* and *X. laevis* rely on speed to evade predation, hence the apparent lack of (or weak) relationship between endurance traits and temperature and are not known to possess good endurance capacity on land (Putnam & Bennett 1981, but see de Villiers & Measey 2017) for overland performance capacity in *X. laevis*). In contrast, *S. capensis* is reliant on toxicity to avoid predation, and is an active forager; hence endurance traits on land are temperature sensitive because they contribute directly to the species' fitness (Taigen & Pough 1983).

Several studies have suggested that performance in anurans is highly influenced by the interplay between ecological specialisation and morphology (Careau et al. 2014; Herrel et al. 2012; Herrel et al. 2014; Hudson et al. 2016; Moen et al. 2013). I expected *X. laevis* to outperform *A. delalandii* at burst swimming performance traits (velocity and acceleration). In contrast, I found that *A. delalandii* consistently outperformed *X. laevis* in burst speed performance traits, both swimming and hopping (Figure 5.1). Considering different performance tests in this study, this result therefore suggests that species that perform well at one trait, may also perform better at other traits such that species that are good jumpers may also be good swimmers (Herrel & Bonneaud 2012b; Herrel et al. 2014; Nauwelaerts et al. 2007; Navas et al. 1999). I also found that T_a had a significant effect on burst hopping velocity for the species considered. Moreover, I found that for burst hopping velocity, all species performed better at intermediate to high temperatures (see Figure 5.4). This result is consistent with the general trend observed for burst swimming velocity results (Figure 5.3). This result may suggest that burst performance among different traits of anurans may be co-adapted (Herrel & Bonneaud 2012b; Huey & Bennett 1987; Huey & Kingsolver 1993).

It is surprising that the mean relative performance for different burst performance traits in this study were largely below the 80% performance threshold (Figure 5.3 and 5.4; see also Huey and Stevenson 1979). This result may suggest that, at least for temperate species living on the edge of their distribution, there is a need to balance the trade-off between performing optimally at narrow environmental temperatures and to perform sub-optimally at a range of temperatures (although see Angilletta et al. 2006; Huey & Hertz 1984). Moreover, in addition to larger performance breadths, evidence suggest that species living in temperate regions will also select for performance at a lower temperature (John-Alder et al. 1988; McCann et al. 2014). That is, in fluctuating environments, some individuals may reach maximum performance for a given trait while most will perform at suboptimal levels (Tingley et al. 2012; Urban et al. 2014; Wilson and Franklin 1999; Wilson et al. 2000). This T_a driven trade-off may be well developed in vertebrate ectotherms such as fishes and amphibians as opposed to reptiles because T_b plays a far more important role in reptiles than in other ectotherms, influencing not only performance but also sex ratios (see Gibbon et al. 2000; Janzen 1994; Shine 2005). In contrast, Hertz et al. (1988) found that in lizards, species frequently maintained close to optimum T_b, although they do not always perform at maximum capacities.

I found that T_a had a significant effect on performance of different frog species. Although this is an important finding given the scarcity of such information, particularly for African anuran species, caution should be exercised when interpreting the results of this study. First, the observed differences could largely be because of phylogenetic divergence between species considered (see chapter four). However, studies which incorporated phylogenetic relationships still found a strong link between performance and ecology (e.g., Moen et al. 2013; Moen et al 2016; Bossuyt and Milankovitch 2000), therefore suggesting that the findings of this study are likely to be stronger than currently presented.

Secondly, the study was designed in such a way that it did not permit the analysis of trade-off within species in a "classical statistical sense" (see Vanhoodonck et al. 2001; Angilletta et al. 2003, Herrel and Bonneaud 2012b and methods for reasons) using the same individuals for each test at each test temperature. The results of this study further suggest that the trade-off between swimming velocity and hopping acceleration may not exist (see above), *A. delalandii* consistently outperformed *X. laevis* in burts performance traits – suggesting that strong jumpers can be strong swimmers.

Future studies looking at the relationship between multiple temperature variables and different performance measures should set up their investigation in such way that it will allow such comparisons although new methods for using different individuals are currently being developed (Careau & Wilson 2017a; Careau & Wilson 2017b). Moreover, studies should also aim at incorporating different life stages as adults may have different physiological limits compared to tadpoles and eggs. They should also include broad thermal spectra to determine the physiological limits, particularly for species with terrestrial adult habits, such as toads, which may experience huge thermal daily fluctuations (Johnson 1972) including life-stages that may not use behavioural thermoregulation such as developing embryos (see Arrighi et al. 2013). And lastly, studies should also focus on disentangling this aspect of thermal performance breadths, while focusing particularly on inter-population differences and thermal reaction norms (Wilson 2001).

In conclusion, this study showed that although temperature is an important variable influencing performance, its impact is more pronounced in terrestrial compared to aquatic performance traits, although I acknowledge that I did not test for swimmimg endurance in this study. For anurans in terrestrial environments, although an increase in temperature leads to increased performance, it also requires increased thermoregulation. This result may have serious implications in the light of the predicted changes in climate, particularly on the terrestrial stages of most amphibians with a bi-phasic life-history. Locally, this may mean that species will be forced to perform at or close to optimal conditions, leading to increased metabolic rate needs and the need to thermoregulate more (see chapter four). Increased metabolic rates also means increased activity during thermally unsuitable periods with detrimental physiological effects, which may be curtailed by the need to thermoregulate. In the long-term, reduced performance in terrestrial activity such as searching for insect prey may lead to a reduction in activity periods and ultimately distribution ranges. Moreover, aquatic environments are known to provide a more thermally buffered environment, where temperature fluctuations are not as pronounced as they may be in terrestrial environments. For burst speed performance traits, *A. delalandii* out-performed *X. laevis* for both burst swimming and hopping performance traits (speed and acceleration), though *X. laevis* has evolved for an aquatic life style (although see Herrel et al. 2016; Measey 2016). This finding further raises the question regarding the evolution of strong-well developed hind legs in *Xenopus* species. Perhaps these strong hind legs (and claws) evolved for feeding as opposed to locomotion (Avila & Frye 1977; Avila & Frye 1978). Moreover, perhaps the *Xenopus* body plan

evolved for aquatic endurance (though this was not tested in this study). Lastly, this result lends support to the suggestion that there should be no trade-off between the two traits as they operate on the same body plan (e.g., Nauwelaerts et al. 2007) such that good swimmers may also be good jumpers. However, good sprinters are generally not good at endurance performance traits and *vise versa* (Calsbeek & Goedert 2017).

Chapter six: Modelling current and future distributions of three temperate African anurans species: an ecophysiology (hybrid) modelling approach

Abstract

Next to habitat loss, global climate change is likely to be one of the leading threats to anuran diversity by the end of the century. To predict how climate change may affect this most threatened vertebrate group, biologists have used correlative species distribution models (SDMs). Despite their versatility, correlative SDMs have been criticised for their simplistic approach of correlating known distributions records with interpolated, coarse scale climate data. Recent advances have advocated for the use of modelling techniques that account for processes such as physiology and performance into SDMs. Here I use ecophysiology modelling techniques to determine the impact of climate on distributions of three African anurans with different ecologies: i) the principally aquatic African clawed frog (*Xenopus laevis*), ii) partially-aquatic common river frog (*Amietia delalandii*) and iii) semi-terrestrial raucous toad (*Sclerophrys capensis*), since the Last Glacial Maximum (LGM ≈ 21 000 YA), under current conditions and how they are expected to change by 2080. I collected data on physiology (standard metabolic rates and evaporative water loss) and performance traits (swimming, hopping & endurance) at different temperatures to account for the effect of temperature variation. The results were used to construct spatially explicit surface models which were used as input layers to model current species distributions using *Maxent*. I then hindcasted these distributions on three past general circulation models (GCMs) during the LGM and then forecasted using the same GCMs for the year 2080. Ecophysiology modelling techniques accurately predicts the distribution of these widely distributed African anurans. Models suggest that anuran species lost thermally suitable space since the LGM, and that the rate of loss between the current conditions and 2080, far exceeds the rate of loss experienced between the LGM and current. Of interest is that the models suggest that *A. delalandii* will gain climatically suitable space by the year 2080, while *S. capensis* is expected to lose suitable climate space in the same period. These results suggest that species may respond to changes in climate individually which will largely be driven by how species adapt to climatic changes at the species-process levels, informed by differences in physiology and performance.

Introduction

As of the year 2015, atmospheric carbon dioxide (CO_2) concentration is now sitting at levels that were last experienced about 4.5 million years ago when global temperatures are thought to have been 4° C warmer than current (Dutton et al. 2015; Pagani et al. 2010). The accumulation of greenhouse gases (e.g., CO_2 and CH_4) resulting from anthropogenic post-industrial revolution activities is singled out as the key driver of the current changes in climate (Jungclaus et al. 2010). Anthropogenic climate change is often generalised as 'global warming' albeit models of future climate suggest far more complex scenarios including changes in diurnal temperature range, cloud cover, precipitation and their consequent interactions (Adler et al. 2008; Zhou et al. 2009). Of note is an increase in the expected frequency of extreme weather events such as heat waves, droughts, floods, storms and cyclones, which may result in disturbance to many ecosystems. Surface air temperatures are also expected to increase continuously towards a warming trend, setting the globe on an irreversible climate change path by as early as the year 2069 (Mora et al. 2013).

To cope with these climatic changes, species have been shown to change their distribution ranges, tracking climatically suitable environments, alter activity times (phenology), behaviour as well as adapting to the changing environment (Davis & Shaw 2001; Parmesan et al. 1999; Parmesan & Yohe 2003). Correlative species distribution models (SDMs) have been extensively used to quantify the effect of climate change on a species' distribution (Araújo et al. 2006; Thuiller et al. 2006) and thus better inform future conservation interventions. Their use has also extended into other disciplines within conservation such as invasion biology (Guisan & Thuiller 2005) and phylogeography (Forester et al. 2013). These correlative SDM approaches make a set of important assumptions: i) species occurrences are in equilibrium with current climate (Araújo & Pearson 2005; Elith & Leathwick 2009) and that: ii) shifts in distribution ranges are but one of the mechanisms that animals can use to counter climatic changes (e.g., phenotypic and behavioural plasticity including adaptation; (see Davis & Shaw 2001). This suggests that distributional changes are largely driven by the inability of species to cope with novel climates (i.e., the concept of conservatism in Grinnellian niche sense, see Chown et al. 2010; Grinnell 1917). This is based on the new set of demands the environment may place on key physiological traits such as energy acquisition, performance and reproduction (see Kearney et al. 2008). However, the two biggest criticisms levelled against correlative SDMs are: i) how they differentiate between realised *vs.* fundamental niche (see Araujo & Guisan 2006) and ii) the use of macroclimatic environmental variables recorded (on average) at 2 metres above ground (e.g., Kearney et al. 2014; Porter et al. 1973) to correlate with known occurrence records may not reflect the microclimatic conditions that particularly lower vertebrates experience.

To account for these species-specific physiological responses, biologists have incorporated physiological responses into methods predicting the impacts of climate change using process-based

models such as mechanistic and ecophysiological models (Buckley et al. 2010; Kearney et al. 2008; Kearney & Porter 2009; Kearney & Porter 2004; Kolbe et al. 2010; Sinervo et al. 2010) and a variation of hybrid models as suggested by Buckley et al. (2011). Mechanistic models aim to reconstruct the microclimate as perceived by the species concerned and then using this microclimate to determine physiologically relevant measures such as estimated hours of activity (h_a), hours of restriction (h_r) and performance across the landscape to estimate vulnerability from climate change (Kearney and Porter 2004). Other process-based methods use broad scale climate data such as WorldClim to estimate h_a, h_r and performance based on known temperature-performance relationships (e.g., Sinervo et al. 2010). Pure mechanistic models use physiological traits to model distributions without correlating spatially implicit physiological layers with known distributions (e.g. Kearney et al. 2008). Hybrid models are a combination of the two types of these major modelling approaches: correlative and process-based models (process based referring to both mechanistic and ecophysiological models; see Buckley et al. 2010, Elith et al. 2010). Hybrid models use the output of process-based models as inputs in a correlative modelling platform (Buckley et al. 2011).

Instead of modelling the probability of occurrence as a function of macroclimatic variables (i.e., the correlative approach), hybrid models predict the probability of occurrence as a function of the specific physiological variable considered (e.g., growth potential, reproduction, metabolic rates), as described by the model threshold (see Sinervo et al. 2010, Ceia-Hasse et al. 2014). Both mechanistic and hybrid models are data intensive, which has limited their use in ecology. However, where comparisons have been made between process-based and correlative-based models, process based models tend to perform better than correlative models (albeit modestly) particularly when projecting distributions to novel, environments (see Buckley et al. 2011, Ceia-Hasse et al. 2014 although see also Kearney et al. 2010). Compared to process-based models, correlative SDMs frequently overestimate the risk of extinction resulting from climate change (Ceia-Hasse et al. 2014; Morin & Thuiller 2009).

Using a spatially explicit ecophysiological model, Sinervo et al. (2010) concluded that approximately 4% of global lizard populations have already gone extinct and further suggested that the extinction rates are expected to increase to about 40% by the year 2080. Kearney et al. (2008) used mechanistic models to determine the fundamental niche of the invasive cane toad in Australia. In this study, the authors mechanistically defined limits of key physiological variables such as terrestrial locomotor performance in adults and the survival of eggs and larval stages in different sized water bodies to map the fundamental niche of the species on the landscape (Kearney et al. 2008). The study concluded that thermal limits on locomotor performance of adults and the limitation on the availability of ponds for larval development set limits for the species invasion under current climate. Although their model incorporates information on different life stages of the cane toad, amphibians represent many other life-history strategies (Duellman 1992) that may be affected differently by predicted changes in climate (Foden et al. 2013).

While most amphibians display a bi-phasic lifestyle, some completely bypass the aquatic larval stage (direct development) while others spend most of their lives in aquatic environments leading to the high variation in life-history traits (Bossuyt & Milinkovitch 2000; Williams & Hero 1998). This variation in life-history traits leads to a variation in ways which species will be affected by threats. Furthermore, evidence also suggests that these differences in ecological specialisations also informs microclimatic specialisations. For instance, studies on rates of evaporative water loss found that species occupying an arboreal habitat have reduced rates of water loss compared to other functional groups (Wygoda 1984), even after accounting for phylogeny (Tracy et al. 2008; Young et al. 2006). These key physiological differences suggests that we are unlikely to have a uniform response to the effects of climate change given the variation in life-history traits in this group of vertebrates although only a few studies have accounted for this variable (see Thuiller et al. 2006). Thus, incorporating physiological traits into species distribution models does present an opportunity to incorporate these fine scale physiological differences that may not be accounted for in traditional correlative SDMs. This will hopefully assist in improving our SDM predictions as we aim to save as much biodiversity as possible from the looming threat of anthropogenic climate change.

Amphibians provide an interesting vertebrate group to assess the impacts of climate change largely because they have poor dispersal abilities (Smith & Green 2005) thus also suggesting reduced ability in tracking climatic changes (Ordonez & Williams 2013). Using spatially explicit ecophysiological hybrid models (Buckley et al. 2011; Elith et al. 2010), the objective of this study is to assess how changes in climate affect distributions of three, widely distributed, largely temperate African anuran species, with different ecologies. Specifically, I want to determine how changes in climate have influenced species distribution across time: from the Last Glacial Maximum (LGM), to current distributions and how these will change under future climate change (see chapters two and three). Therefore, the aim of this study was to first determine how species distributions have shifted over time (Mokhatla et al. 2015). Secondly, to estimate how distribution range sizes are likely to change as a function of climate. Moreover, seeing that anurans have poor dispersal abilities, I also wanted to determine how fragmentation affects frog distributions as climates change. Lastly, using different general circulation models, I aim to determine the influence of climatic variability on estimates of extinction threat (e.g., Thuiller 2004).

Methods

Species capture and collection

To meet the study aims, I selected three different frog species based on their apparent dependence on water. I chose the principally-aquatic African clawed frog (*Xenopus laevis*), ii) stream-breeding common river frog (*Amietia delalandii*), and iii) the principally terrestrial raucous toad (*Sclerophrys*

capensis). I caught between 20 and 30 adult individuals (of varying but overlapping interspecific body size classes) of each of the three species around Port Elizabeth, South Africa, deliberately excluding juveniles and gravid females. Both male and female individuals were used in this study to account for any sex-specific size differences from sexual dimorphism. Animals were kept for a maximum four months under environmentally enriched laboratory conditions (see below), until all experiments were completed. *Amietia delalandii* were maintained in 110 L plastic boxes, with sand and small logs for cover, at low densities (5 individuals per box). *Sclerophrys capensis* were kept in terraria made from circular paddling pools (*d x h*: 2.16m × 0.45m - ≈ 1 600 L capacity) with sand, water and small logs and bark to provide cover. *Xenopus laevis* were kept in a freshwater tank (*l x b x h*: 3.55m × 0.90m × 0.63m) with stacked-bricks and stones to provide adequate cover and was fed a diet of ox heart. Both *Amietia quecketti* and *Sclerophrys capensis* were fed mealworms and crickets, dusted with calcium (ReptiCalcium, Zoo Med Laboratories Inc; South Africa). All species had food available *ad libitum*, and the holding rooms were maintained at 20° C, on a 12:12 photoperiod. Atmospheric air was circulated to maintain a constant temperature into the holding rooms, thus ambient humidity levels fluctuated with outside air. After completing the experiments, animals were returned to their respective sites of capture.

Physiological data

The central tenet of this thesis is understanding how changes in temperature affect physiology and performance, and how these thermal responses inform species distributions. Thus, I incorporated physiological and performance variables that reflect the energy and mass balance, though at varying degrees of importance. Thus, the final list of physiological and performance traits had to be sensitive to changes in temperature. According to Buckley et al. (2010), these variables can be classified as life-history models as they use relationships between environment and performance to define fitness across a range of specified environments (across the entire study area). Physiological variables considered for this study were standard metabolic rate and evaporative water loss. These variables give an indication of how distinct species use energy and balance water loss, a critical aspect in amphibian biology (e.g., Wygoda 1984). For performance, I chose traits that give an indication of speed: velocity, acceleration (both swimming and hopping). These traits are important in evading predation and catching prey. Lastly, I conducted endurance (hopping distance and time until exhaustion) experiments to make inferences about dispersal abilities (see de Villiers & Measey 2017; Kearney et al. 2008; Phillips et al. 2010; Phillips et al. 2006).

I measured standard metabolic rates (SMR) and evaporative water loss (EWL) following Lighton (2008) and Steyermark et al. (2005) using an open-flow respirometry system operated on a push through

mechanism on post-absorptive, non-reproductive individuals, at rest (Sinclair et al. 2013). Experiments were conducted at temperatures ranging from 5°C to 35°C at 5°C intervals (Dunlap 1971). Eight individuals of each species (4 males: 4 females) were randomly selected for trials at each temperature and a single trial was undertaken on each individual per temperature (not replicated a number of times since these experiments are time and labour intensive). Frogs were placed individually in suitably-sized air tight glass metabolic chambers. Experiments were carried out using Mass Flow System pumps (MFS-2, Sable Systems, Las Vegas, NV) to pump atmospheric air scrubbed of water vapour using a Drierite column (98 % $CaSO_4$, 2% $CoCl_2$, Sigma-Aldrich, South Africa) at a flow-rates of 100 to 600 $mL.min^{-1}$, through the two metabolic chambers: one metabolic chamber with a frog and the other recording baseline conditions. Air from the metabolic chambers was sequentially sub-sampled, using Subsampler (SS3, Sable Systems, Las Vegas, NV) and a Multiplexer (V3, Sable Systems) was programmed though Expedata (Sable Systems, Las Vegas, NV) to record gas concentrations for each chamber at 20 minute intervals, recording an air sample every second. Subsampled air was first pulled through a water vapour analyser (RH-300, Sable Systems, Las Vegas, NV) to measure water vapour pressure. The behaviour of the frogs was monitored during the trials using live video feed for the duration of the trial. A trial was considered completed when the water vapour pressure and temperature trace was stable for 20 minutes or when the animal appeared too distressed to continue with measurements. Trials did not last longer than 2 hours. For detailed methods, see chapter four.

Performance

Burst swimming and hopping speed

Burst speed performance traits (burst swimming and burst hopping) were measured by randomly selecting 10 individuals (5 males and 5 females), but ensuring minimal over-exertion on individuals. Experiments were conducted in a temperature controlled room, at a range of environmental temperatures, from 10° to 30°C at 5°C intervals (Herrel & Bonneaud 2012a; Herrel & Bonneaud 2012b; Wilson et al. 2000). Target test temperatures within climate rooms were set overnight before commencing with experiments and I proceeded only when the temperature was within 1° C of the targeted test temperature. This was achieved by reconciling the temperature on the mercury thermometer in the climate room with the temperature recorded using a thermocouple probe (Fluke 54IIB, Fluke Corporation, Washington). Test temperatures were also selected at random to minimise any effect of a directional change in temperature on performance traits. Prior to experimentation, animals were placed for one hour in individual containers with water in an incubator set at the target temperature (Navas 1996). All performance measurements were repeated twice for each individual with an inter-trial period of approximately an hour, during which animals were returned to the incubator to be maintained at the target temperature, and allowed to rest. Trials were conducted in a clear Perspex

tank ($l \times b \times h$: 2m × 0.4m × 0.4m), marked at 25 cm intervals. I mounted a mirror (1.4m × 0.4m) above the tank, at a 45° angle, to ensure that during trials, animals were visible both from the dorsal and lateral views. Only the best view was used during analysis (see below).

Burst of speed performance was recorded using a high speed digital video camera (Canon *PowerShot* G15, Canon Inc., Japan) set at 240 frames per second (*fps*). Swimming and hopping burst speed was initiated by a light tap on the urostyle. The video clips were then processed and cropped to include at least two locomotor cycles and analysed manually. For each frame, the snout tip was digitized and tracked across all cropped frames that successfully captured the identified two locomotor cycles, using an image tracking software Blender (version 2.74; Blender Foundation, Amsterdam). The coordinates of the animals' movement were exported to Excel (Microsoft, Redmond, WA, USA) and the displacement of the individual along its path was calculated. The raw displacement profile was then smoothed by using a zero phase shift low-pass Butterworth filter version 2 with the cut-off frequency set at 30Hz, adapted from (Winter 2005). The final maximum velocity and acceleration were calculated by numerical differentiation of the smoothed displacement profiles. For each individual, I extracted the highest instantaneous velocity and acceleration across all swimming sequences recorded: i.e. the fastest velocity and acceleration may thus come from different sprint swimming performance (Herrel & Bonneaud 2012a; Herrel & Bonneaud 2012b; Herrel et al. 2012; Herrel et al. 2014; Miller 1982). Refer to chapter five for detailed methods.

Maximum exertion

Six individuals of each species (3 males and 3 females) were randomly selected for endurance experiments. To determine this, I chased an individual animal along a 4.7 m circular track until exhaustion. This was expressed as the point at which the individual was not able to right itself when placed on its back (see Herrel & Bonneaud 2012a, Herrel & Bonneaud 2012b). Experiments were only undertaken on a dry surface (maximal hopping exertion) with the floor of the track covered with a high friction matting (GECKO® Non-slip matting, Cape Town) to improve traction. Mass was recorded to the nearest ± 0.01g before and after the experimental run. I also ensured that body temperature (T_b) was within 1°C of the target T_a before trial by recording the cloacal temperature using a thermocouple probe (Fluke 54IIB, Fluke Corporation, Washington). For each individual, I recorded both the total distance covered and the time spent moving until exhaustion (Herrel & Bonneaud 2012a).

Ecophysiology modelling

Key to constructing ecophysiological (hybrid) and mechanistic models is perhaps the reconstruction of microclimate surfaces as experienced by species of interest, particularly ectothermic vertebrates

(amphibian and retiles; see Porter et al. 1973, Kearney et al. 2014). Although there are similarities in the data that is incorporated in the modelling processes, there are also a few fundamental differences between the two processes-based approaches. First, both methods use largely similar environmental data input (see Buckley et al. 2010). In addition to similar environmental temperature variables (i.e., minimum, average, maximum temperatures, precipitation and altitude; see Buckley et al. 2011, Sinervo et al. 2010), mechanistic models may also include cloud cover, radiation, and wind speed (Porter et al. 1973 and more recently Kearney et al. 2014). Second, mechanistic models construct these microclimate conditions without incorporating species distribution records (e.g., Kearney et al. 2008 using data from all life stages), while distribution records are included in the ecophysiological (hybrid) process to both project distributions (see Buckley et al. 2011) and crop the surface models (Barry Sinervo *pers comm*). Third, both models use (mainly physiologically) derived thresholds to determine species range margins (e.g., Buckley et al. 2010; but see Sinervo et al. 2010; Clusella-Trullas & Chown 2011). Lastly, both models also predict how performance varies across space and these performance outputs have been used as inputs for correlative species models in a hybrid approach: mechanistic (e.g., Elith et al. 2010) and ecophysiological (e.g., Buckley et al. 2011).

In this study, I follow Buckley's (2011) approach by using performance derived geographical surface models to model the distributions of three widely occurring, yet sympatric frog species in southern Africa. This is largely because I did not record behavioural data (see Porter et al. 1973) either in the lab or in the natural environment to set physiologically relevant thresholds for the other two outputs (h_a and h_r see below). Moreover, when dealing with species with complex life-cycles such as frogs, factors that may seem to be limiting in the adult stage may not be important when considering other thresholds associated with different life stages (see Kearney et al. 2008).

Geographical surfaces for ecophysiological predictors (see Ceia-Hasse et al. 2014, Sinervo et al. 2010) were built with insight from the temperature-performance relationship in Mapinguari v0.4.1, implemented in R v3.3.2 (R Development Core Team 2017). In addition to the temperatire-performance relationships obtained in different chapter, I also included information on the themal minimum and maximum for each of the species and performance variabes involved based on observations and literature (e.g., Vimercati 2017; Vimercati et al 2018; Wilson et al. 2000). I included information on Snout-Vent-Length for each individual frog. I then modelled the relationship between temperature and physiology/performance using General Additive Mixed Models (GAMM), by fiting equation to the data. These GAMM models were used as inputs to determine surface area models (see below). These models assume that performance is a proxy for demography, such that species would generally occupy niches that support a positive net energy balance that enables performance, growth, development and reproduction (Kearney and Porter 2004). To construct these geographical surface models across the entire distribution range of the target species, climate data was obtained from WorldClim (Hijmans et al. 2005) at 2.5 arc minutes spatial resolution. Climate data included 19 bioclimatic variables, altitude,

precipitation and different monthly temperature variables (*viz*., minimum, mean and maximum) under current climate conditions (1950-2000). To compute surface area models, I used temperature-performance/physiology relationship (i.e., thermal performance curve) to estimate performance, h_a and h_r, in each grid, given the set of environmental variables in that grid (*please note that h_a and h_r,are also standard output of the models*). In addition, I wanted to determine how changes in climate have influenced the distributions of these widely occurring species across different time periods.

I also constructed these ecophysiological surface models under both past (LGM ≈ 21 KYA) and future (by the year 2080) climate conditions using three general circulation models (GCMs) to account for climate uncertainty. The GCMs that were considered for the LGM were: the Community Climate System Model (CCSM: (Otto-Bliesner et al. 2006), the Model for Interdisciplinary Research on Climate (MIROC: (Hasumi & Emori 2004), and the Max-Planck Institut für Meteorologie (MPI-ESM-P: (Giorgetta et al. 2013). Paleo-climate data were downscaled to a spatial resolution of 2.5 arc minutes following the delta method (Peterson & Nyari 2008). I also used similar GCMs to construct future ecophysiological surface models under the RCP 8.5 climate scenario which represents the highest greenhouse emissions, while assuming high human population growth, slow income growth, and modest technological advances (Riahi et al. 2011). These future GCMs included the CCSM4 (Gent et al. 2011), MIROC-ESM (Watanabe et al. 2011) and the MPI-ESM-LR (Block & Mauritsen 2013). The final output of each physiological variable surface model included the thermal performance (see supplementary figure S6.1 to 6.4), hours of activity (h_a: cumulative hours in a day when T_b is within the range of lowest physiobiological relevant T_b and T_{pref}) and hours of restriction (h_r: cumulative hours each day when $T_e > T_{opt}$; where T_e = environmental temperature) geographical surface maps. Inter-correlation structure among predictors (performance surface area model output, see supplementary figure S6.1 to S6.4) throughout the study area was assessed by computing pairwise squared Spearman's rank correlation coefficients, and in those cases where R^2 exceeded 0.75, only the putatively biologically most important variables were retained.

Occurrence data

I used the most recent taxonomic treatment of the target species (Frost 2017) especially as two of the three species have recently undergone taxonomic revisions (Channing et al. 2016; Ohler & Dubois 2016), although see Furman et al. 2015 for *X. laevis*). Occurrence records were obtained from the South African Frog Atlas Project (Minter et al. 2004), CapeNature, I-Spot, published literature (e.g., De Busschere et al. 2016; Ihlow et al. 2016; Measey & Channing 2003) including records from several South African Natural History museums and G.J Measey's *X. laevis* distribution records. I only incorporated records with accuracy to the nearest second or higher. For *Xenopus laevis* I used 789 records, *A. delalandii* 1038 records and *S. capensis* 728 records. Distribution records were projected

onto Arc Map GIS 10.3 (ESRI 2014) to identify any doubtful records that may be recorded outside known ranges, using Global Amphibian Assessment (GAA 2008, IUCN; http://www.iucnredlist.org/ accessed July 2017) polygons as 'know distribution ranges'. These were used to judge whether the outlying records represented credible sightings or should be excluded from further analyses.

Species Distribution Modelling

Species distribution model projections were created at a 2.5 arc minute resolution, using the machine learning algorithm *Maxent* v3.3.3k (Phillips & Dudík 2008), based on the thermal performance layers obtained above. *Maxent* has been successfully used to model past and current distributions of endemic southern African frog species (Schreiner et al. 2013). For model evaluation, I divided the species records into 80% used to train the models and 20% for model evaluation using the area under the receiver operating characteristic curve: AUC (Swets 1988). The final model was averaged over 100 replicates, using the bootstrap sampling technique. A circular buffer of 500 km around each locality point was selected as the training area, following Measey et al (2012). When projecting the ensemble across space and time, non-analogous climatic conditions that exceeded the training range of the models were quantified wherein the numbers of these predictors are highlighted using Multivariate Environmental Similarity Surfaces (MESS: Elith et al. 2011). I used the "Minimum training presence threshold" referring to the lowest generated probability estimates of the training data (Pearson et al. 2007) to convert logistic probabilities of occurrence to binary predictions (presence/absence), with suitable predictions predicted above the threshold and unsuitable predictions below the threshold (see also Ihlow et al. 2016). I used these binary predictions output in subsequent analysis.

SDM changes across time

To assess changes in species distributions across climatic periods, I used the binary prediction outputs as the final models. I then determined the level of overlap between distribution ranges across time, with the LGM distributions as the baseline by comparing the Schoener's *D* (dismo package: Hijmans et al. 2015) in R. To explore how individual species distributions change across time, I determined the species geometric means, using the 'sp'package (Pebesma & Bivand 2005) see also (Duan et al. 2016) where a similar method was followed. The distance between the geometric means was used the determine biotic velocity between different time periods (see chapter 1). Using PatchStat function from the SDMTools package (VanDerWal et al. 2014), I calculated the total area, perimeter and shape index to determine the fragmentation index across time (i.e., Landscape Dissection Index or shape index, DI´ following (Bowen & Burgess 1981). This was achieved by determining the edge of each species distribution range, in relation to the core of the distribution using the formula:

$$DI' = \sum_{i=1}^{n} P/(2\sqrt{\pi \sum_{i=1}^{n} A})$$ equation 1

where DI´ is Landscape Dissection Index, *P* is Perimeter and *A* is the Area; DI´ compares the amount by which any "island" or a distribution range is dissected relative to the total area, with a DI´ of 1 representing a circle, suggesting a less fragmented shape with a low Perimeter to Area ratio. Previous studies have suggested that species in southern Africa are expected to shift their distributions on both north and eastward directions (see Chapter two; Erasmus et al. 2002). Botts et al. (2015) suggets that anuran species in the region have already shifted to higher altitude areas as well as in a nortwestawd direction, particularly in the last 100 years.

Results

Model fit and projections

I found that the hybrid-based *Maxent* models accurately described the distributions of the three widely distributed species with good statistical fit 0.825 (AUC_{test}) and 0.826 ($AUC_{training}$) for *A. delalandii*, 0.817 (AUC_{test}) 0.818 ($AUC_{training}$) for *S. capensis*, and 0.816 (AUC_{test}) and 0.816 ($AUC_{training}$) for *X. laevis*. Rates of EWL contributed significantly (98.2%) to the *A. delalandii* model, followed by hopping acceleration (1.4%) and endurance time (0.4%). For *S. capensis*, endurance time contributed 72.8% to the model, followed by EWL (25.9%) and hopping acceleration (1.2%). Rates of EWL contributed 86.2% to the *X. laevis* model, followed by endurance time (8.8%) and hopping velocity (4.9%).

For *A. delalandii* I found that predictions could be projected to all the GCMs considered in the study (Figure 6.1 A). The projections of current distributions accurately match what we know as current distributions based on distributions records (Figure 6.1 A, 6.1 B and 6.1 C). Moreover, areas along the Orange River valley, the Lowveld (the low-lying north-eastern part of South Africa and Swaziland) and other dry areas in the Karoo, are also not identified as suitable despite the occurrence of these synathropic species in these regions, mediated by recent human modifications such as the developments of farm dams, which may have created suitable habitat for these species (Measey et al. 2017). For *X. laevis* specifically, the model does not identify the interior of Botswana as suitable habitat despite known species records. In contrast to known distribution records, the models identify suitable habitats for all three species on the south-western part of Namibia, for *S. capensis* and along the length of the coast for both *A. delalandii* and *X. laevis*. However, I could not project distributions for the MIROC based GCMs both in the last glacial maximum, and for the year 2080 for *S. capensis* (Figure 6.1 B), and MIROC 2080 for *X. laevis* (Figure 6.1 C). This suggests that climatic conditions for these GCMs may represent non-analogous climatic conditions that may perhaps be outside the bounds of one or a

combination of the final physiology/performance measures considered (see MESS and see also Fitzpatrick & Hargrove 2009; Zurell et al. 2012).

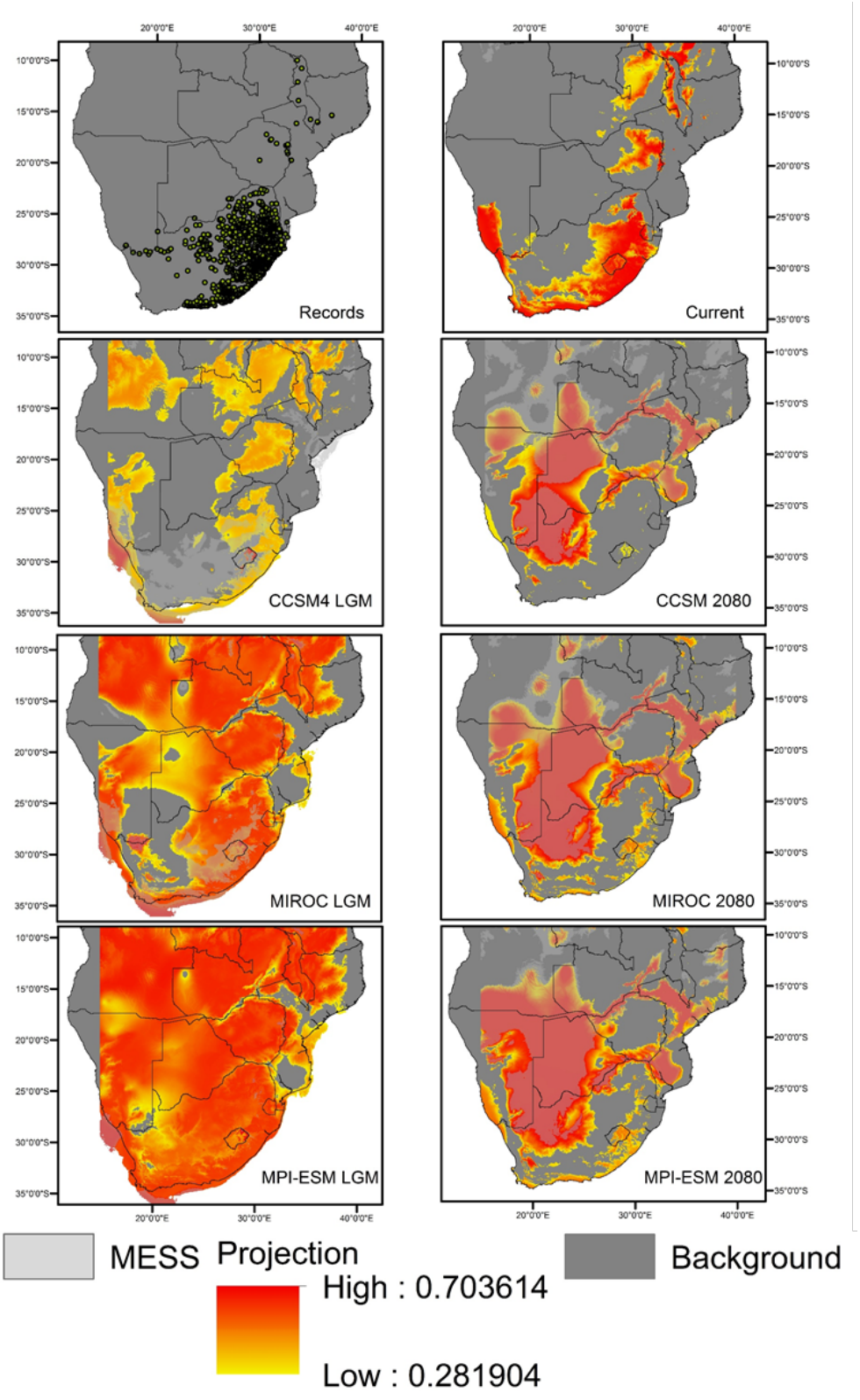

Figure 6.1 A. *Amietia delalandii* distribution records and projections from the last glacial maximum, current through to predicted projection for the year 2080 using different GCMs

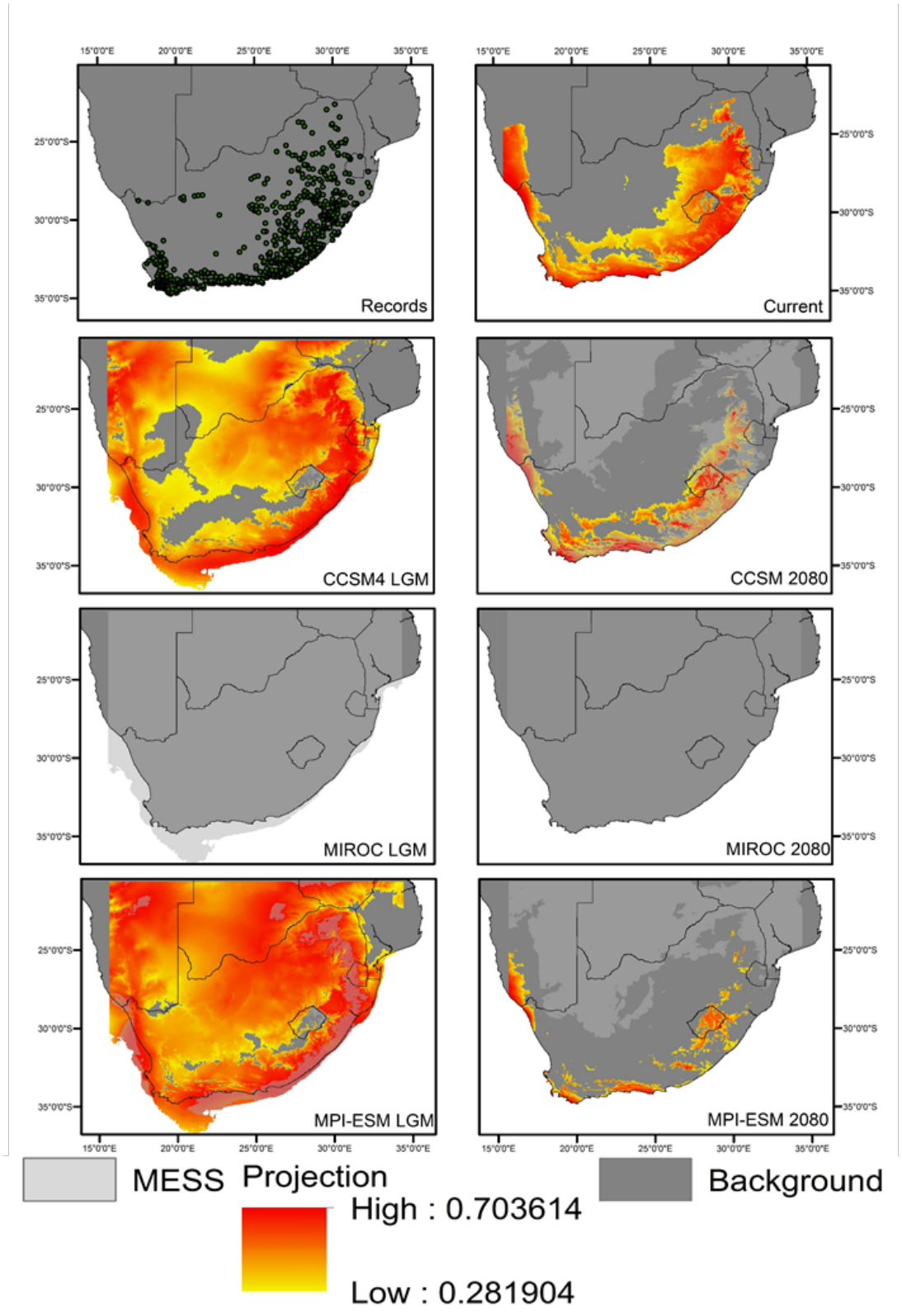

Figure 6.1 B. *Sclerophrys capensis* distribution records and projections from the last glacial maximum, current through to predicted projection for the year 2080 using different GCMs.

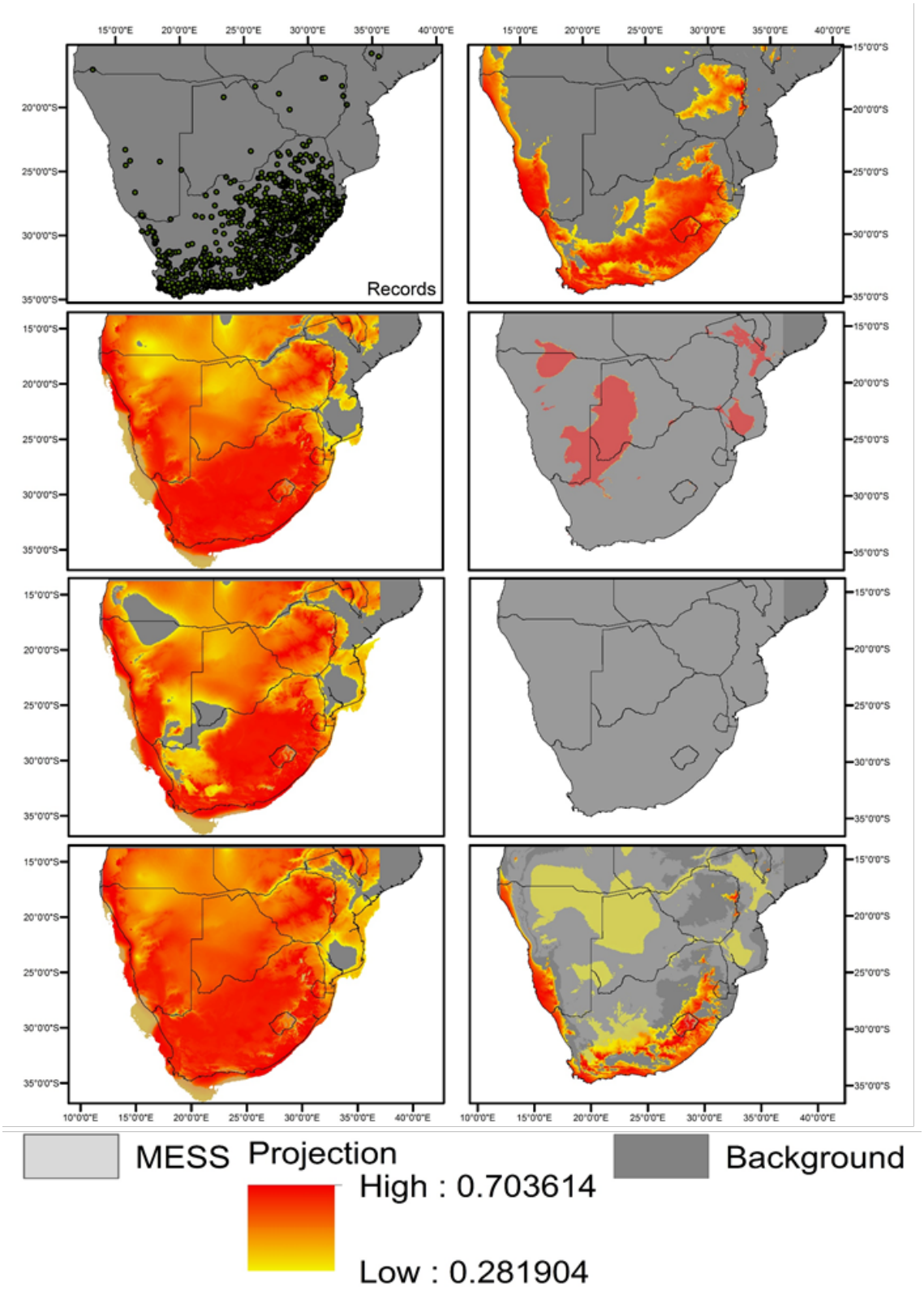

Figure 6.1 C. *Xenopus laevis* distribution records and projections from the last glacial maximum, current through to predicted projection for the year 2080 using different GCMs.

Distribution range changes

I found that between the LGM and current, the models predict that all species considered, irrespective GCM, experienced reduction in distribution ranges (see Figure 6.2). The models suggest that *A. delalandii* lost between 40 and 71% of suitable area during this period. *Sclerophrys capensis* and *X. laevis* are predicted to have lost between 64 to 70% and 68 to 70% of suitable area, respectively. The models also predict that *S. capensis*, when considering both MIROC and MPI-ESM-P GCMs, is expected to experience further reduction of distribution ranges: 43% loss in suitable area for the CCSM GCM and 78% loss when considering the MPI-ESM GCM (Figure 6.2 B). Of the two GCMs that projected suitability for *X. laevis* in 2080, only the CCSM GCM predicts further reduction in distribution range of *X. laevis* between current conditions and future projections (2080: Figure 6.2 C), which equates to the loss of 53% of the currently suitable area. *Amietia delalandii* on the other hand is expected to expand its distribution range between current and 2080 for all three GCMs (Figure 6.2 A) by between 47 to 95% in suitable area.

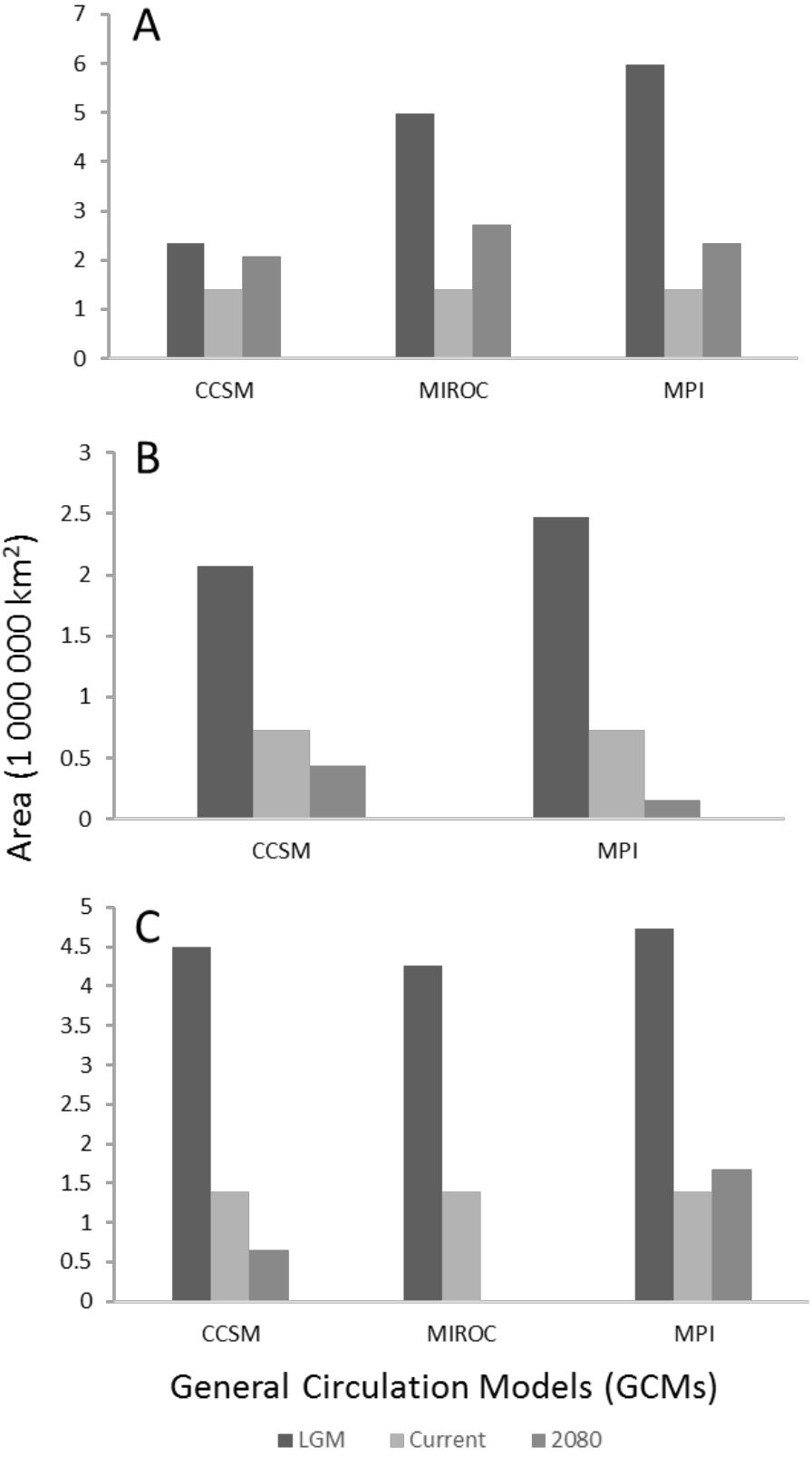

Figure 6.2. Predicted distribution range sizes using different GCMs. A = *Amietia delalandii*; B = *Sclerophrys capensis*; and C = *Xenopus laevis*

Landscape dissection index

Landscape dissection index generally increased from the LGM to current conditions as well as between current and predicted distributions although this was only found for *S. capensis* across all GCMs (Figure 6.3). There were however deviations from this expected trend for *A. delalandii* and *X. laevis*. For *A. delalandii*, I found that the landscape dissection index increased between the LGM and current, and between current and future predicted distributions for the year 2080. This result was observed when considering projections from CCSM GCM only. For *X. laevis*, I found that a decrease in landscape dissection index between current and future predicted distribution for the CCSM GCM (see Figure 6.3).

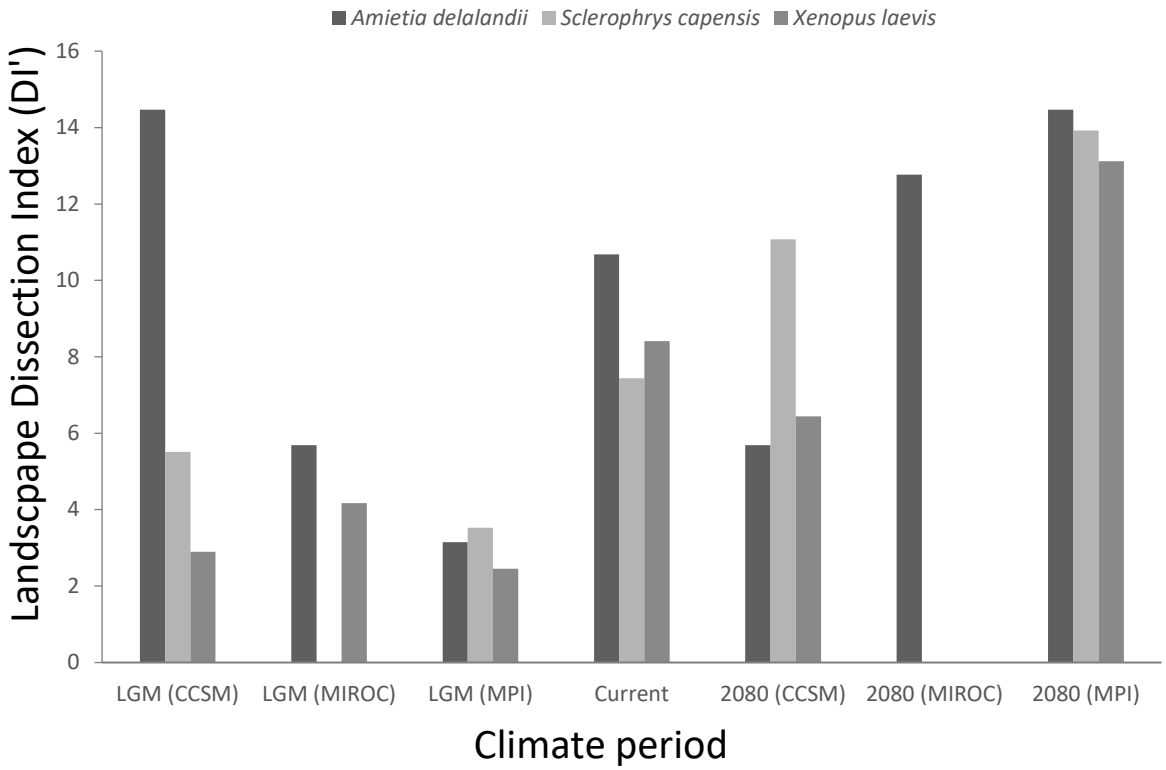

Figure 6.3. Landscape dissection index (DI') for different frog species across climatic periods.

***Movement across time* (*biotic velocity*)**

Model projections suggest a southward directional shift between the LGM and current for *A. delalandii* at the rate of 0.05 km. yr^{-1} (for both MIROC and MPI GCMs) to keep up with predicted changes in thermal environment. For CCSM GCM, the model predicts that *A. delalandii* tracked suitable thermal environment at the rate of 0.01 km. yr^{-1} in a south-easterly direction. For *S. capensis*, the models predict that the core of the distribution shifted in the south-eastern direction at the rate of 0.01 km. yr^{-1} (for both CCSM and MPI GCMs) between the LGM and Current. The *Xenopus laevis* model predictions suggest

that the distribution of the species shifted southward between the LGM and current conditions at the rate of 0.03 km. yr^{-1} for all three GCMs.

Future projections for *A. delalandii* suggest that distribution ranges are generally expected to shift between current conditions and 2080 in a north-westerly direction at the rate of 10.2 $km.yr^{-1}$and 11.0 $km.yr^{-1}$ under CCSM and MPI GCMs, respectively. Results from the MIROC GCM predicts that *A. delalandii* is expected to shift northwards at the rate of 11.2 $km.yr^{-1}$ to track thermally suitable areas between current conditions and the year 2080. *Xenopus laevis* is also expected to shift its distribution northwards at the rate of 14.0 $km.yr^{-1}$ and for MPI GSM and in a north-western direction, at the rate of 6.5 $km.yr^{-1}$ for CCSM GSM. To track thermally suitable areas by the year 2080, *S. capensis* is expected to further move towards the southeast direction at the rate of 1.9 $km.yr^{-1}$ under CCSM and Eastern direction at the rate of 4.4 $km.yr^{-1}$ under MPI GSM (see also Figure 6.4).

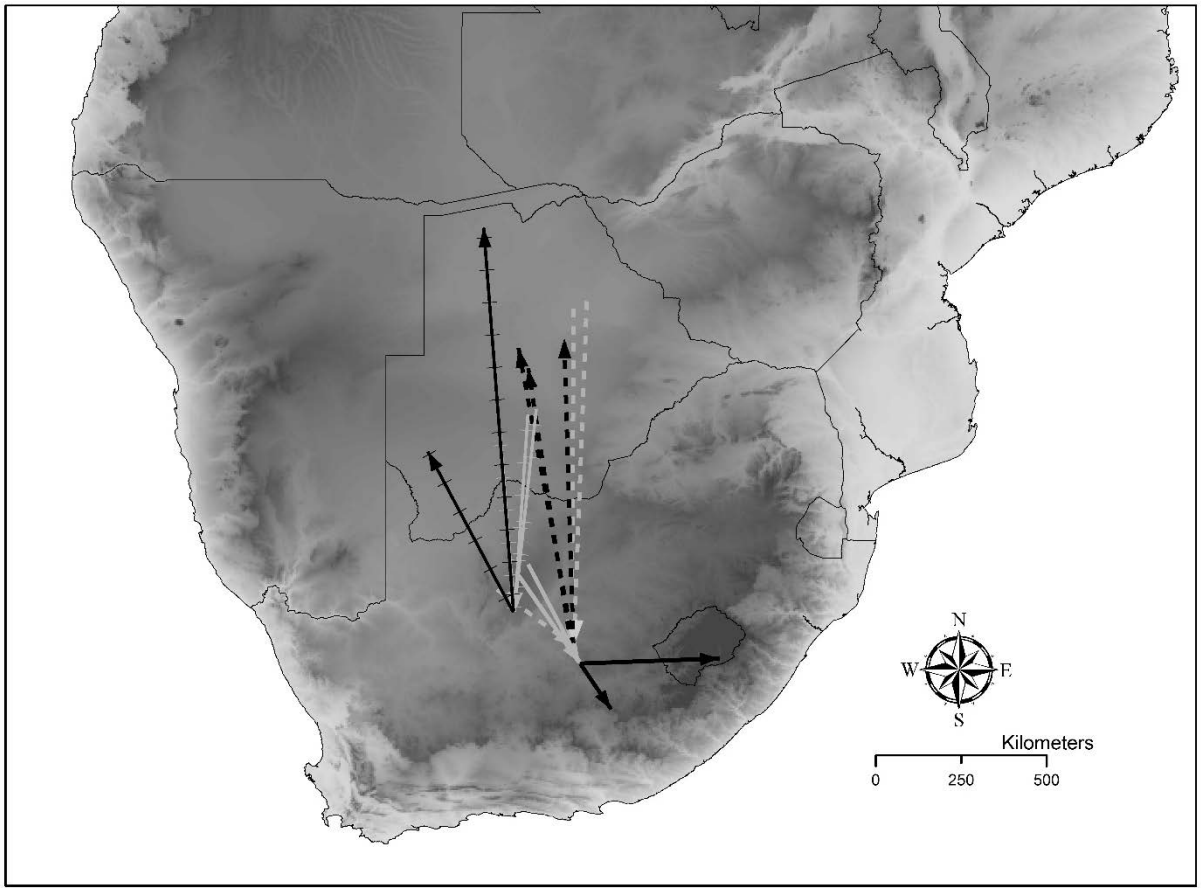

Figure 6.4. Predicted changes in distribution ranges (biotic velocity) across different climatic periods using the different GCMs. The arrows represent distance and direction of species range shifts at different climatic periods, grey arrows represent predicted biotic velocity between the LGM and Current, black arrows denotes predicted biotic velocity between Current and 2080. Dashed arrows = *Amietia delalandii*, solid arrows = *Sclerophrys capensis* and marked arrows = *Xenopus laevis*.

Discussion

This study shows that changes in climate between the LGM and current conditions may have led to similar species responses, loss of suitable thermal space, the general slow rates of biotic velocity and the southwards movement in distribution centres. On the contrary, future climatic change is expected to lead to a diversity of responses in temperate African anuran species with different ecologies. *Amietia delalandii* is expected to gain in thermally suitable space across all GCMs while *S. capensis* is expected to lose thermally suitable space. Rate of biotic velocity is expected to be far higher than historical rates, if species are to track thermally suitable habitats. Fragmentation (as determined by the Landscape Dissection Index) between the current and future, far exceed the ratio between LGM and current.

Current thermal niches as projected in this study more accurately match the distribution records when compared to other methods of describing current species distributions (e.g., IUCN polygons, Channing et al. 2016). For *X. laevis*, these include published expert drawn distributions (e.g.,(Furman et al. 2015) and modelled current fundamental niches: (Ihlow et al. 2016; Measey et al. 2012; Rödder et al. 2017). The fundamental niche (i.e., Grinnellian niche) defined by most of these methods (excluding Measey et al. 2012- which focused on modelling the distribution of the Cape clade only) identify *X. laevis* as occupying a larger area than records can currently support. These methods use a combination of temperature and precipitation variable (e.g., WorldClim) to estimate the species fundamental niche. In this study, I used the temperature and physiology/performance relationship to determine where species may be able to perform different physiologically pertinent tasks such as the cost of self-maintenance and dispersal. This result perhaps lends support to Poynton's proposition outlining the role of temperature as the primary variable explaining large-scale distribution patterns in anurans (Poynton 1964a; Poynton 1964b), with rainfall influencing patterns more at a local scale within the broadly defined temperature region. These results further suggest that incorporating physiological information into SDMs lead to more accurate distribution predictions (Nowakowski et al. 2017; Peterman & Semlitsch 2014; Riddell et al. 2017), particularly for amphibians.

Changes in climate have been attributed as one of the defining features of the distribution of anurans in southern Africa since the LGM (Alexander et al. 2004; Poynton 1964a; Poynton 1964b; Schreiner et al. 2013). Evidence to this effect is seen from the occurrence of what Poynton termed the transitional fauna of the central region of southern Africa, which he suggested is a relic of past climatically-induced contractions and expansions of the 'tropical' and 'Cape fauna' when climates were suitable (Alexander et al. 2004, Poynton 1964b, Schreiner et al. 2013). In this study, I found that between the LGM and current conditions, the rate at which species had to move to thermally suitable habitats to keep up with climate change (i.e., biotic velocity, see Ordonez and Williams 2013) has been between 0.01 $km.yr^{-1}$ to 0.05 $km.yr^{-1}$ for all three species (see also Chapter two). Range expansions and retractions into refugia during periods of change have also been observed elsewhere in the world, and these climate refugia

have been characterised by both stable climate during the late quaternary period and topographic heterogeneity (Araújo et al. 2008; Baselga et al. 2012; Cowling et al. 2009). This relatively slow rate of climate change has also been attributed as the reason why these areas are rich in range restricted, endemic species (Dynesius & Jansson 2000; Sandel et al. 2011). In addition, to stable climates, these regions also had high topographic variation at short spatial scales (Cowling et al. 2009). While studying the fossil amphibian community of Langebaanweg in the early Ploicene (5.1 Mya), (Matthews et al. 2016; Matthews et al. 2015) found the remains of two anuran families that are not represented on the winter rainfall region under current climate conditions, suggesting that in addition to shifting distributions of species, past climatic changes possibly led to novel species interactions as species moved. This suggests large distribution range shifts before the Quaternary period but does not exclude that these species may have gone extinct in these areas in the process. This is perhaps something that could be avoided under future climate change (through better conservation management planning and interventions). Such range expansions and contractions generally occur at a species level since species differ in their respective thermal tolerances (Deutsch et al. 2008).

Under future climate change, species would need to track thermally suitable habitat by as much as between 2.0 $km.yr^{-1}$ to 14.0 $km.yr^{-1}$ to keep up with climate change in this century. This suggest anuran species require higher biotic velocities when comparing outputs of the two modelling processes (see chapter three). In contrast, to areas that have experienced stable climates in the past (see above); areas that have experienced pronounced climate change velocity in the past are suggested to harbour species with large distribution ranges, poor in endemic species and topographically invariable (see Sandel et al. 2011). I found that species did not show a specific directional (poleward) shift. Furthermore, I found that *A. delalandii* (under all GCMs) and *X. laevis* (under the MPI GCM) are expected to gain distribution ranges. Given the historical climate and the large distribution ranges of these Afro-temperate species (see Poynton 1962), it may have been possible for these species to occupy most thermally suitable habitats by 2080 if it was not of the extent to which current habitat are transformed (Measey et al. 2017). However, the Schroener's *D* value suggests that the level of similarities between distributions occupied at under current and 2080, particularly for *A. delalandii* is generally low (see Table 1). Such range expansions may perhaps be far-fetched, given the limited dispersal abilities of most anurans, particularly under very such transformed landscapes as well as the role that extreme climatic periods (e.g., prolonged droughts) may play as climates change.

In a recent analysis, Botts et al. (2012) found that as much as 60% of South African anuran species have lost suitable climate space over the last 100 years. Two of the species in this study are also confirmed to have suffered recent range contractions, and only *X. laevis* is confirmed as having experienced range expansions in the same period (see Botts et al. 2012, Supplementary Information). These species range losses have been attributed largely to habitat modification in the region which is also suggested to have had an opposite effect on other largely synanthopic species such as *X. laevis* (Measey et al. 2017). In

addition to *X. laevis*, evidence further suggests that other species such as the painted reed frog (*Hyperolius marmoratus*) have also used human transformed landscapes (e.g., farm dams) to increase their south-western distribution range margin further westwards into the winter rainfall region of the western cape province (Davies et al. 2013; Tolley et al. 2007). However, the Schroener's *D* value suggests that the level of similarities between distributions occupied at under current and 2080, particularly for *A. delalandii* is generally low (see Table 6.1). This further suggests that such range expansions may perhaps be impossible, given the that these areas currently present non-anologue climates as these areas are currently drier compared areas that the species currently occupy, although see Engelbrecht et al. (2011).

Table 6.1. Niche overlap calculated as Schoener's *D* between baseline different time periods, using the LGM distribution as baseline. Numbers closer to one represents little or no change in niche space, while numbers close to 0 a near total niche shift.

	Amietia delalandii	*Sclerophrys capensis*	*Xenopus laevis*
LGM (CCSM) - Current	0.68	0.88	0.90
Current - 2080 (CCSM)	0.19	0.90	0.98
LGM (MIROC) - Current	0.64	-	0.91
Current - 2080 (MIROC)	0.28	-	-
LGM (MPI) – Current	0.61	0.88	0.89
Current - 2080 (MPI)	0.29	0.89	0.91

Although there are predicted increases is suitable areas for some temperate, synanthropic anurans, Deutsch et al. (2008) concluded that climate change will have a devastating effect on tropical species with small range sizes as these species occur in areas that are close to their thermal optima. This is because T_{max} does not seem to vary across latitudes and that temperate species have been able to occupy temperate regions by lowering their CT_{min} (Duarte et al. 2012; Gutiérrez-Pesquera et al. 2016). Contrary to Deutsch et al. (2008), this study predicts that *S. capensis* may disappear from some of its thermally suitable areas between current and 2080 for both CCSM and MPI GCMs and *X. laevis* under CCSM GCM. This could be because temperatures in some of *S. capensis* range may be expected to go above the current thermal safety margins (see Gerick et al. 2014), or that ectotherms in general are predicted to occupy much as 18% of the thermally suitable areas under future climate change perhaps leading to further fragmented distributions (Buckley et al. 2013).

Since the LGM, fragmentation has also increased between the two climate periods for all species (Figure 6.3). Other past climatic models suggest amphibians as a class, are not in equilibrium with current climate conditions, such that current amphibian distributions only occupy between 30 and 60% of their potential fundamental niches (Munguía et al. 2012). This mismatch between amphibian distributions

and current climates has been identified as one of the reasons why amphibians in general have small distribution ranges which also further reduces their changes of being represented in conservation networks (Rodrigues et al. 2004), and further suggests that we may have been underestimating the threat that amphibians face from climate change. This expected fragmentation resulting from range reductions as anuran species lose thermally suitable areas may thus lead to more isolated populations, particularly if taken in the context of the combined effect changes in land-use and climate change as the most dominant threat expected to impact African anurans (see Hof et al. 2011, Garcia et al. 2014, although see also Nowakowski et al. 2017).

Although a powerful technique, ecophysiology models have also been met with criticism particularly with extrapolation to other regions and species (Clusella-Trullas & Chown 2011). This however does not take away from the assertion that this technique is robust when paired with adequate data of the same species within the same region. However, the results obtained under these modelling techniques should also be interpreted with caution. Firstly, despite all the measures taken to reduce the likelihood of projecting species distribution to areas that may lack current climate analogues (such as clamping and MESS, see Zurrell et al. 2012), models still project species on these areas, for instance in the dry central interior of Botswana and Namibia (see Figure 5.1 A). Perhaps this is because I used only temperature related variables (temperature-performance relationship). I acknowledge that the inclusion of precipitation as a variable, or the distribution of waterbodies may have improved future projections. Secondly, models do not account for acclimation, which is suggested to be very important in frogs to cope with both short and long term directional shifts in temperature (see chapter 3, (Orizaola & Laurila 2016), although also see (Telemeco et al. 2017). Thirdly, it is also crucial to remember that the physiological measurements/parameters were measured in the lab under controlled environments, and may thus not reflect field performances. Collecting field behavioural data could have improved the data, particularly in setting threshold limits (see Kearney et al. 2008 on cane toads). Lastly, although describing the temperature-performance relationship is critical in building process-based models, it should be noted that I sampled single populations and phenotypic change throughout the species range may play a large part in determining adaptation (see below). Furthermore, I also acknowledge that these temperature-performance relationship does not remain constant throughout ontogeny more so for groups with complex life cycles as frogs (Sinclair et al. 2016).

In this study, I have shown that the inclusion of broad-spectrum temperature-performance relationships on key physiological and performance variables into SDMs can not only improve our understanding of current species distribution ranges, but also further help understand how distinct species (based on different ecologies), may respond to predicted changes in climate. The inclusion of all biologically relevant temperature (Kearney et al. 2008) assisted models not only to identify areas where performance may not be possible (i.e., CT_{max}, see Sinervo et al. 2010) or areas where performance is only optimal (Kearney and Porter 2004). This is largely because performance between different traits is not co-

adapted (see chapter four) and that ectotherms have evolved to occur and function under less optimal conditions (see chapter five). Thus, where possible I would advocate for the use of more than one physiology/performance variable, across a range of biologically relevant temperatures. In addition to incorporating different life stages, particularly when studying species with complex life cycles such as anurans, future studies should also consider accounting for both genetic variation (Ikeda et al. 2017) and local adaptation (Orizaola & Laurila 2016) by incorporating individuals from different populations across the species range into SDM models. I believe that this will bring us a step closer to more accurate species extinction risk assessments and enable us to save as many species as possible, from global change factors affecting most of biodiversity now.

Chapter seven: Correlative *vs* ecophysiology modelling?

Conclusion

In this PhD thesis, I used correlative-based SDM techniques to model distribution ranges of endemic anurans of the CFR characterised by stable climate throughout the quaternary period. I hindcasted these distributions to two past climatic periods (i.e., LGM and HGM) and then forecasted these distributions to the year 2080 to determine how changes in climate may have structured the distribution of these narrowly distributed endemics in the past and in the foreseeable future. I then used the same modelling techniques to construct distribution models for three temperate African anuran species, with different ecological specialisations: i) the principally-aquatic African clawed frog (*Xenopus laevis*), the semi-aquatic, stream-breeding common river frog (*Amietia delalandii*), and the largely terrestrial raucous toad (*Sclerophrys capensis*). These three species have a different climate history compared to the CFR endemic group in that they occupy an area that has experienced climatic changes throughout time as global climates were changing (Alexander et al. 2004). My results suggest that the impact has been more pronounced on the CFR anuran community as compared to the widely distributed temperate species of the transitional fauna - such that the CFR group has lost approximately 56 % of suitable climate since the LGM despite experiencing relatively stable climate, while the transitional fauna has lost between 30 and 40 % of suitable climate in the same period. Although species representing both groups may experience habitat loss by 2080, the biggest loss is without a doubt on the CFR anurans. In addition to currently occupying restricted ranges, by 2080 the CFR endemic group is also expected to lose between 60 and 70 % of suitable climate space, while the transitional species are expected to lose between 20 and 50 % of suitable climate space. What is particularly interesting for one of the transitional species; *A. delalandii* is that all future models, irrespective of GCM used, suggest that the species will gain suitable climate space suggesting that species will respond individually to the challenges posed by climate change in the future, mediated by the diversity in functional traits, particularly in anurans.

In chapter three, I looked at the impact of temperature and body mass in influencing key physiological factors such as body temperature, standard metabolic rates and evaporative water loss. I found that temperature had a significant effect on key physiology traits. Interestingly, I also found that body temperature increased with an increase in body mass only in *A. delalandii* and *S. capensis*. This result seems to suggest that the importance of body mass on the heat flux of anurans may be crucial for species that spend a proportional amount of time on land, where changes in temperature may be more pronounced as opposed to species that spend most of their time within thermally stable aquatic environments.

Chapter four looked at the how the variation in temperature affects different performance traits in the three species representing different ecologies. The importance of temperature in influencing performance was more prominent in terrestrial performance traits, thus highlighting the importance of thermoregulation and the need for improved performance at high environmental temperatures, while

also managing high rates of evaporative water loss. Temperature did not have a significant impact on performance trade-offs between burst swimming *vs*. hopping acceleration. This result may suggest that species that are better at hopping acceleration may also be excellent swimmers. However, this result may not be sustained such that jumping would trade-off against hopping endurance while burst swimming would trade-off against endurance swimming. The importance of temperature in influencing terrestrial performance traits in different species may lead to reduced performance in terrestrial activity such as searching for insect prey, dispersal and ultimately distribution ranges of these species.

The main aim of the thesis was to revitalise SDM techniques by incorporating performance, morphology and physiology into modelling process by constructing ecophysiology (hybrid) models. Since there is a general lack of such data for most African anuran species, I collected data on the effect of temperature on physiology (standard metabolic rates and evaporative water loss) and performance: burst swimming speed (acceleration and velocity), burst hopping speed (acceleration and velocity) and hopping endurance (distance and time). I then incorporated these data into ecophysiology modelling techniques. I used these traits (performance, physiology and morphology) to build surface models that were incorporated as inputs into a correlative-based modelling process. This was achieved by integrating physiology, morphology and performance data into the modelling process by building surface models. Based on the physiology/performance *vs*. temperature relationship, surface model outputs highlight areas where performance is possible while assuming performance as a proxy for demography. These surface models were used as inputs in a *Maxent* modelling process to build more accurate ecophysological models for the three widely distributed, Afro-temperate anuran species. Although changes in temperature have led to similar responses since the LGM, species are expected to be impacted differently by anthropogenic-induced changes in climate owing to the predicted increase in suitable climate space for *A. delalandii*, while both *S. capensis* and *X. laevis* are expected to lose suitable climate space. I found that the inclusion of broad-spectrum temperature-performance relationships on key physiological and performance variables into SDMs can not only improve our understanding of current species distribution ranges, but also further help understand how distinct species (based on different ecologies), may respond to predicted changes in climate. Since performance is not co-adapted in anurans, the models were also improved by including all relevant temperatures so that we not only identify areas where performance may not be possible (see Sinervo et al. 2010) or areas where performance is only optimal (see Kearney and Porter 2004).

When looking at the predicted areas from both modelling techniques (i.e., chapters three & six), correlative modelling consistently predicts smaller distribution ranges (binary outputs) compared to areas predicted by the ecophysiology technique. Moreover, the rate at which species are expected to track suitable climate is relatively high for ecophysiology model outputs compared to correlative-based models. This may suggest that we are underpredicting the impact of climate change on African anurans. This thesis highlights that not all anurans are equal in the face of predicted climate change, irrespective

of the modelling technique used. Moreover, both climate history and ecological specialisation are important variables to consider if we are to correctly predict the impact of future climate change. This study further draws our attention to the consideration that understanding key trait relationships with temperature may assist us to correctly identify species that may be more at risk resulting from climate change. In addition, this technique provides important biological meaning into how changes in climate may affect species distribution and the mechanisms involved in the process.

However, the data intensive nature of these models (correlative models: 4 days to run all 37 CFR endemics, 1 day to run models for the three Afro-temperate species; ecophysiology models: ± 4 months of physiology and performance data collection, 2 to 3 months of extracting performance profiles, 3 months of computation time to build surface area models (on an average computer), plus half a day to run ecophysiology models in *Maxent*) has largely precluded their use in ecological studies. Moreover, I do acknowledge both the consideration of phylogenetically unrelated species and the use of one species representing each functional group limited the generality of the results observed, particularly in chapter four and five. Moreover, the specimens collected in this study constitute data from a single population per species and thus do not take local adaptation and climate sensitivity into consideration when projecting when constructing surface area models (e.g., Amburgey et al. 2018). However, conducting the relevant physiological, behavioural, performance, morphology and modelling on representative groups throughout the distribution of any widespread species, should be the subject of several PhD studies (or a consortia of academics) rather than a single PhD.

I suggest that, ideally for widely distributed species, these models should be considered and in instances where we need to understand the impact of climate change on rare, narrowly distributed species, the use of correlative based SDMs techniques is advocated. The information gathered on physiology and other relevant traits, may also be used to ask other pertinent questions that will assist us in understanding the mechanism of temperature-performance relationships (see chapter four and five). Despite its shortcomings, this thesis should be a welcomed investment seeing that we lack such basic data for most African anuran species. In addition, future studies should consider using phylogenetically related species and aim to have a broader representation in each functional group as well as consider sampling different populations across the species range to account for broader representation of climate regimes and genes. More in-depth investigations on the performance-temperature relationship such as this, will further enhance our understanding at identifying largely terrestrial, low-altitude anurans species currently occupying fragmented distributions (due to human land-use) that may be more vulnerable to the impacts of climate change in the future.

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Supplementary Information

Supplementary table S2.1: Cape Floristic Region endemic amphibian species grouped according to Family, IUCN category, life-history trait and altitudinal specialisation

Species name	Family	IUCN category	Egg deposition and development groups	Altitude
Afrixalus knysnae	Hyperoliidae	Endangered	mode18	Lowland
Amietia vandijki	Pyxicephalidae	Data Deficient	mode1	Highland
Sclerophrys pantherina	Bufonidae	Endangered	mode1	Lowland
Sclerophrys pardalis	Bufonidae	Least Concern	mode1	Lowland
Arthroleptella bicolor	Pyxicephalidae	Least Concern	mode17	Highland
Arthroleptella drewesii	Pyxicephalidae	Data Deficient	mode17	Highland
Arthroleptella landdrosia	Pyxicephalidae	Near Threatened	mode17	Highland
Arthroleptella lightfooti	Pyxicephalidae	Near Threatened	mode17	Lowland
Arthroleptella rugosa	Pyxicephalidae	Critically Endangered	mode17	Lowland
Arthroleptella subvoce	Pyxicephalidae	Endangered	mode17	Highland
Arthroleptella villiersi	Pyxicephalidae	Least Concern	mode17	Highland
Breviceps acutirostris	Breviceptidae	Least Concern	mode17	Highland
Breviceps fuscus	Breviceptidae	Least Concern	mode17	Lowland
Breviceps gibbosus	Breviceptidae	Vulnerable	mode17	Lowland
Breviceps macrops	Breviceptidae	Vulnerable	mode17	Lowland
Breviceps montanus	Breviceptidae	Least Concern	mode17	Lowland
Breviceps namaquensis	Breviceptidae	Least Concern	mode17	Lowland
Cacosternum capense	Pyxicephalidae	Vulnerable	mode1	Lowland
Cacosternum karooicum	Pyxicephalidae	Data Deficient	mode1	Highland
Cacosternum namaquense	Pyxicephalidae	Least Concern	mode1	Highland
Cacosternum platys	Pyxicephalidae	Least Concern	mode1	Lowland
Capensibufo rosei	Bufonidae	Vulnerable	mode1	Highland
Capensibufo tradouwi	Bufonidae	Least Concern	mode1	Highland
Heleophryne hewitti	Heleophrynidae	Critically Endangered	mode2	Lowland
Heleophryne orientalis	Heleophrynidae	Least Concern	mode2	Lowland

Species name	Family	IUCN category	Egg deposition and development groups	Altitude
Heleophryne purcelli	Heleophrynidae	Least Concern	mode2	Highland
Heleophryne regis	Heleophrynidae	Least Concern	mode2	Lowland
Heleophryne rosei	Heleophrynidae	Critically Endangered	mode2	Lowland
Hyperolius horstockii	Hyperoliidae	Vulnerable	mode18	Lowland
Microbatrachella capensis	Pyxicephalidae	Critically Endangered	mode1	Lowland
Poyntonia paludicola	Pyxicephalidae	Near Threatened	mode1	Highland
Strongylopus bonaespei	Pyxicephalidae	Least Concern	mode13	Highland
Strongylopus springbokensis	Pyxicephalidae	Vulnerable	mode13	Highland
Tomopterna delalandii	Pyxicephalidae	Least Concern	mode1	Lowland
Vandijkophrynus angusticeps	Bufonidae	Least Concern	mode1	Highland
Vandijkophrynus robinsoni	Bufonidae	Least Concern	mode1	Highland
Xenopus gilli	Pipidae	Endangered	mode1	Lowland

Supplementary table S2.2: Percentage contribution of final set of bioclimatic predictors used after determining the inter-correlation structure of 19 initial variables

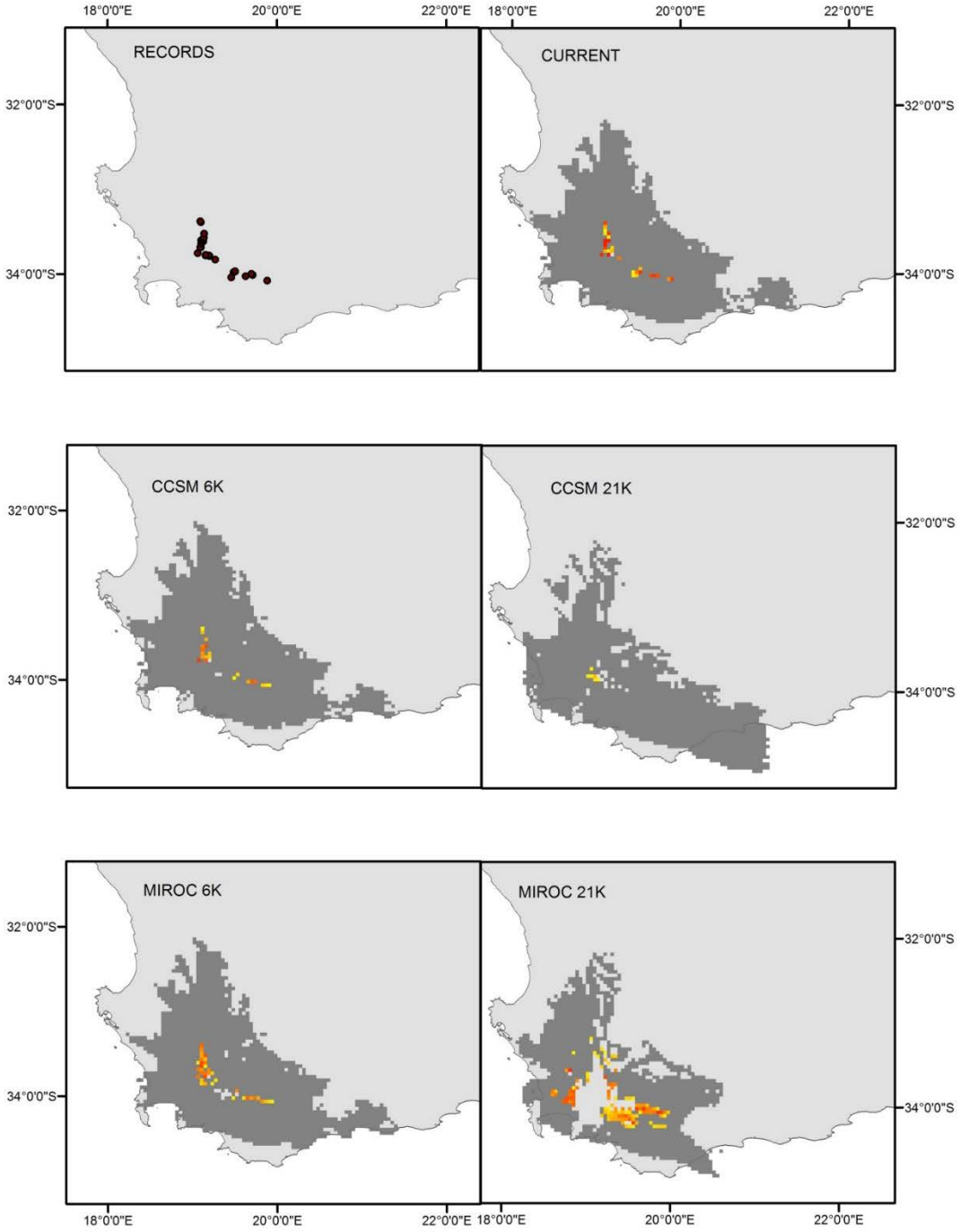
Species	BIO10	BIO11	BIO13	BIO15	BIO17	BIO19	BIO3	BIO7	BIO8	BIO9
Afrixalus knysnae	7.743991	10.67119	14.3908	8.302279	11.936	13.72584	3.61571	14.46007	7.124749	8.029369
Amietia vandijki	17.39621	11.7875	9.669936	10.48824	13.11202	11.89466	6.872975	8.364904	4.991738	5.4218172
Sclerophrys pantherina	7.805023	15.88895	12.91163	6.802673	7.670527	19.0036	2.600731	12.14429	6.162754	9.0098253
Sclerophrys pardalis	11.5093	10.8487	9.167925	12.87207	12.51675	11.91126	5.205984	7.003782	6.522108	12.442123
Arthroleptella bicolor	9.593349	15.68784	11.36405	9.505886	15.0933	10.08614	6.465105	8.637043	6.878256	6.6890372
Arthroleptella drewesii	12.54954	6.915392	14.26171	14.35277	12.08398	15.37238	5.216042	10.14865	2.822844	6.2766926
Arthroleptella landdrosia	11.23956	13.96334	8.472137	12.0075	12.90638	9.233001	6.649601	9.655311	9.536168	6.3369981
Arthroleptella lightfooti	9.159001	6.269758	8.414263	4.176516	12.66482	19.05989	6.229886	21.60311	3.212485	9.210264
Arthroleptella rugosa	8.219165	8.299981	11.63075	8.766596	9.186069	11.27862	13.807	13.14701	7.635174	8.0296325
Arthroleptella subvoce	6.85449	12.79548	10.68707	11.98805	15.05	12.49134	4.115075	10.07771	7.68573	8.2550437
Arthroleptella villiersi	8.581717	11.51247	8.470914	12.00443	13.35069	10.13629	9.807202	10.37784	6.00554	9.7529086
Breviceps acutirostris	17.12028	9.944252	9.098796	9.93576	12.96242	10.92705	4.649634	9.053386	6.970391	9.3380344
Breviceps fuscus	9.410888	10.00848	7.712176	9.319536	13.581	12.9834	9.492996	10.87741	6.937315	9.6767879
Breviceps gibbosus	11.99317	11.82804	15.90028	7.977323	7.941763	10.24663	4.697616	11.33469	7.905386	10.175103
Breviceps macrops	12.02958	10.37779	8.558153	10.38996	6.115747	8.767363	15.32464	12.31056	6.345635	9.7805727
Breviceps montanus	12.41313	10.94425	9.098796	9.93576	12.96242	12.92705	4.649634	10.05339	7.677591	9.3380344
Breviceps namaquensis	10.97418	10.54389	10.30435	7.621773	10.58291	13.60148	7.282522	8.984197	8.84221	11.262492
Cacosternum capense	10.92778	9.303938	14.2227	6.500397	10.58814	9.572234	8.317172	8.103138	8.874866	13.589638
Cacosternum karooicum	9.841953	9.639938	10.74904	12.04431	13.36401	9.853836	7.262633	10.04859	9.221385	7.9743058
Cacosternum namaquense	8.452222	7.714444	14.75333	11.47667	9.856667	9.958889	7.681111	11.24	8.722222	10.144444
Cacosternum platys	16.11706	19.74557	6.099815	7.098588	9.11788	7.934264	5.981764	8.668976	10.17723	9.0588546
Capensibufo rosei	12.64284	8.556219	14.92428	8.332386	7.174805	9.265742	8.549163	13.73848	7.807888	9.0081968
Capensibufo tradouwi	10.94857	17.11476	7.518095	8.477619	10.68905	8.968571	11.44048	9.875714	7.72619	7.2409524
Heleophryne hewitti	12.82873	3.649309	7.024947	8.076017	11.98918	18.21056	6.112347	16.33957	7.221205	8.5481268
Heleophryne orientalis	14.59641	11.79025	10.08834	9.854428	11.28482	11.46847	5.655867	12.76073	6.335837	6.1648553
Heleophryne purcelli	12.61414	16.95195	8.33475	8.565762	8.216629	16.60586	4.725291	11.80778	6.054702	6.1231339
Heleophryne regis	8.30741	9.345133	10.25496	8.766069	14.25968	12.98771	8.529478	12.01885	6.835388	8.6953263
Heleophryne rosei	8.418422	10.64921	7.005147	3.950221	30.67999	15.13231	4.554256	7.542986	3.836034	8.2314197

Species	BIO10	BIO11	BIO13	BIO15	BIO17	BIO19	BIO3	BIO7	BIO8	BIO9
Hyperolius horstockii	12.08828	11.51129	9.304369	8.985437	6.82694	6.540524	10.14148	18.52779	6.047944	10.025943
Microbatrachella capensis	8.626956	8.124454	13.81088	11.43938	8.258521	12.25669	14.65897	10.87431	5.976404	5.973425
Poyntonia paludicola	14.29412	10.64127	9.247158	10.00252	10.80411	12.75398	7.215347	10.03194	8.910974	6.0985859
Strongylopus bonaespei	9.218761	7.844743	15.25777	11.52598	11.61697	13.84634	5.846907	7.431223	9.536231	7.8750746
Strongylopus springbokensis	10.27146	8.848929	9.496718	9.104145	6.991789	13.24869	11.20913	10.40406	11.09214	9.3329289
Tomopterna delalandii	8.912257	6.837648	6.452227	6.318995	10.59669	31.56643	3.149981	13.52303	5.186525	7.4562238
Vandijkophrynus angusticeps	7.456201	5.850223	10.73342	9.747509	16.74682	18.55204	5.853659	13.52113	5.314325	6.2246651
Vandijkophrynus robinsoni	9.739188	7.401116	9.353274	7.739896	10.43933	9.250167	12.17644	13.50308	12.3748	8.0227032
Xenopus gilli	7.526525	12.74192	10.71201	9.417674	10.37676	15.22866	7.530209	12.87332	5.147853	8.445083

Supplementary table S2.3: Ensemble model accuracy score

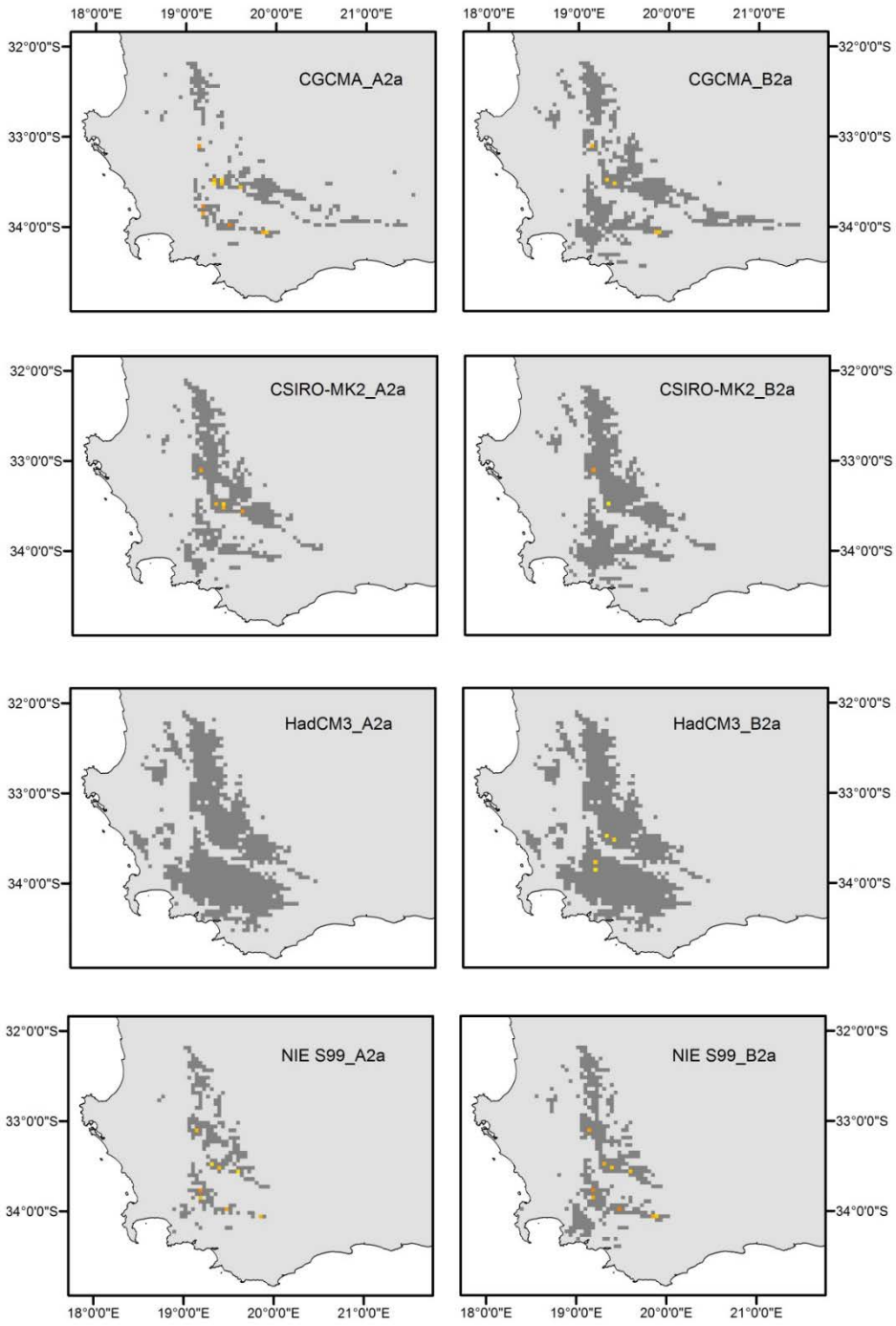
	Kappa	TSS	ROC
Afrixalus knysnae	0.669	0.975	1
Amietia vandijki	0.556	0.969	0.999
Sclerophrys pantherina	0.946	0.955	0.998
Sclerophrys pardalis	0.52	0.817	0.966
Arthroleptella bicolor	0.679	0.976	1
Arthroleptella drewesii	1	1	1
Arthroleptella landdrosia	0.972	0.976	0.999
Arthroleptella lightfooti	0.974	0.959	0.998
Arthroleptella rugosa	0.454	0.964	1
Arthroleptella subvoce	0.352	0.96	1
Arthroleptella villiersi	0.84	0.919	0.992
Breviceps acutirostris	0.686	0.947	0.995
Breviceps fuscus	0.553	0.897	0.986
Breviceps gibbosus	0.636	0.924	0.992
Breviceps macrops	0.637	0.973	0.998
Breviceps montanus	0.723	0.978	0.998
Breviceps namaquensis	0.353	0.872	0.964
Cacosternum capense	0.505	0.8	0.954
Cacosternum karooicum	0.306	0.959	0.997
Cacosternum namaquense	0.392	0.715	0.928
Cacosternum platys	0.289	0.957	1
Capensibufo rosei	0.654	0.952	0.995
Capensibufo tradouwi	0.519	0.917	0.99
Heleophryne hewitti	0.987	0.989	1
Heleophryne orientalis	0.647	0.971	0.997
Heleophryne purcelli	0.695	0.809	0.967
Heleophryne regis	0.848	0.898	0.989
Heleophryne rosei	1	1	1
Hyperolius horstockii	0.648	0.806	0.968
Microbatrachella capensis	0.967	0.992	1
Poyntonia paludicola	0.877	0.982	0.998
Strongylopus bonaespei	0.581	0.808	0.957
Strongylopus springbokensis	0.459	0.966	0.995
Tomopterna delalandii	0.471	0.566	0.854
Vandijkophrynus angusticeps	0.48	0.686	0.918
Vandijkophrynus robinsoni	0.404	0.777	0.95
Xenopus gilli	0.641	0.974	0.999

Supplementary figure S2.1: Distribution records, current (6 Kya) and the two paleoclimate (6 and 21 Kya) projection scenarios for *Arthroleptella bicolor* projected using two different general circulation models (CCSM and MIROC).



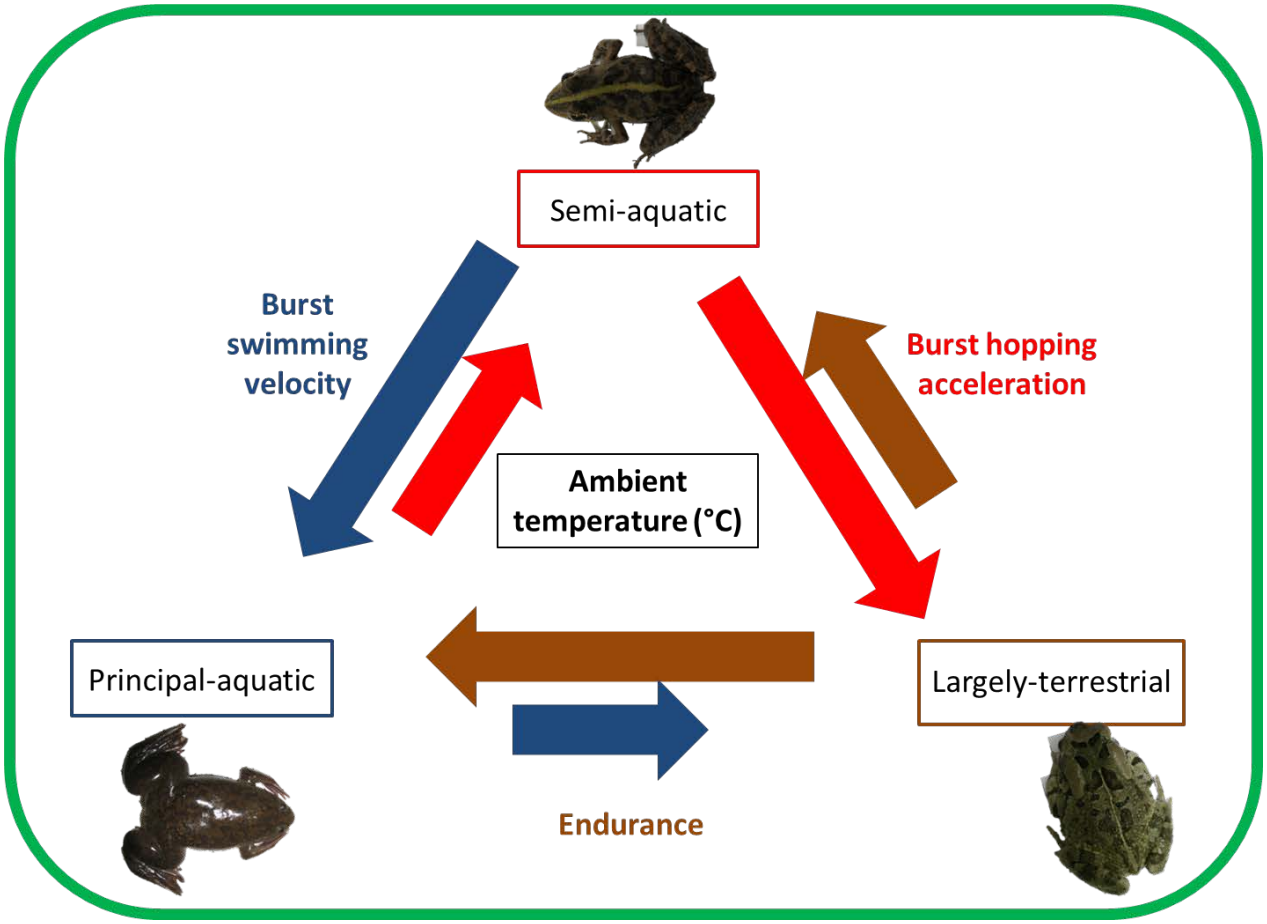
Supplementary figure S2.1

Supplementary figure S2.2: Future (2080) climate projections for *Arthroleptella bicolor* projected using four different general circulation models (CGCMA2, CSIRO-MK2, HadCM3 and NIE S99) and two Intergovernmental Panel on Climate Change emission scenarios (A2a and B2a).



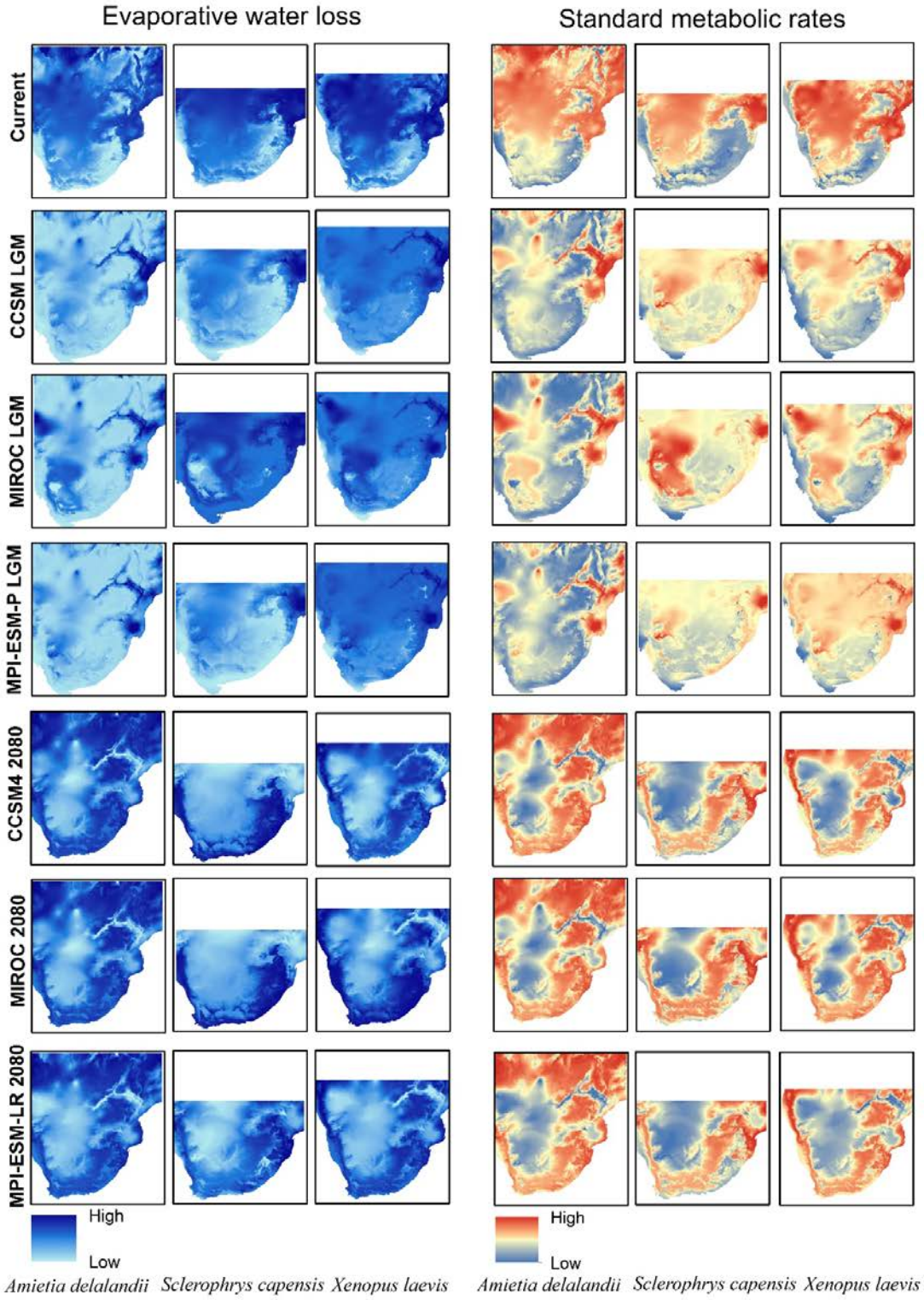
Supplementary figure S2.2

Supplementary figure S5.1: A representation of the proposed way in which species pairs were hypothesised to out-perform each other.



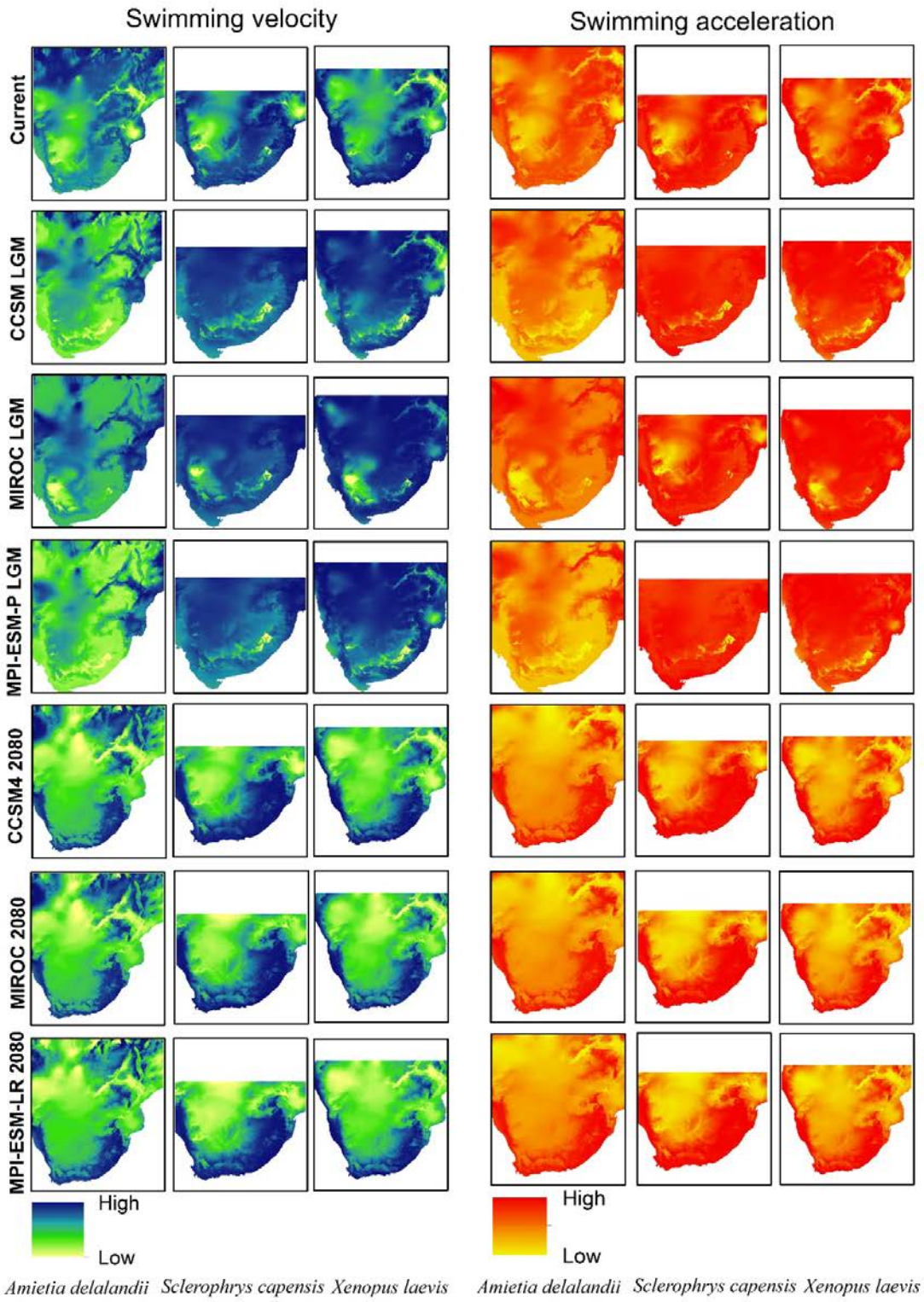
Supplementary figure S5.1

Supplementary information S6.1: Surface models constructed from EWL and SMR thermal performance



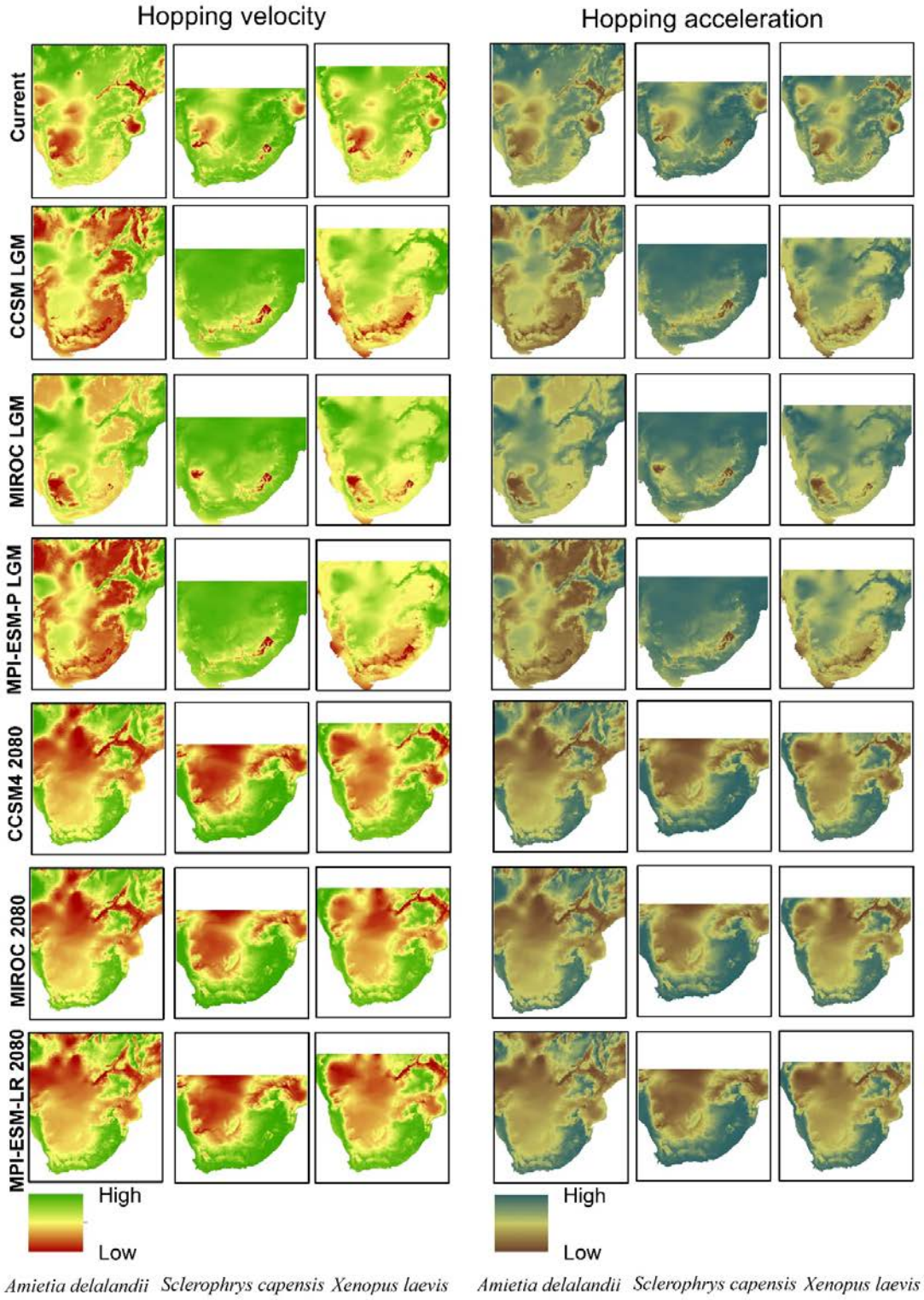
Supplementary information S6.1.

Supplementary information S6.2: Surface models constructed from swimming velocity and acceleration thermal performance measures.



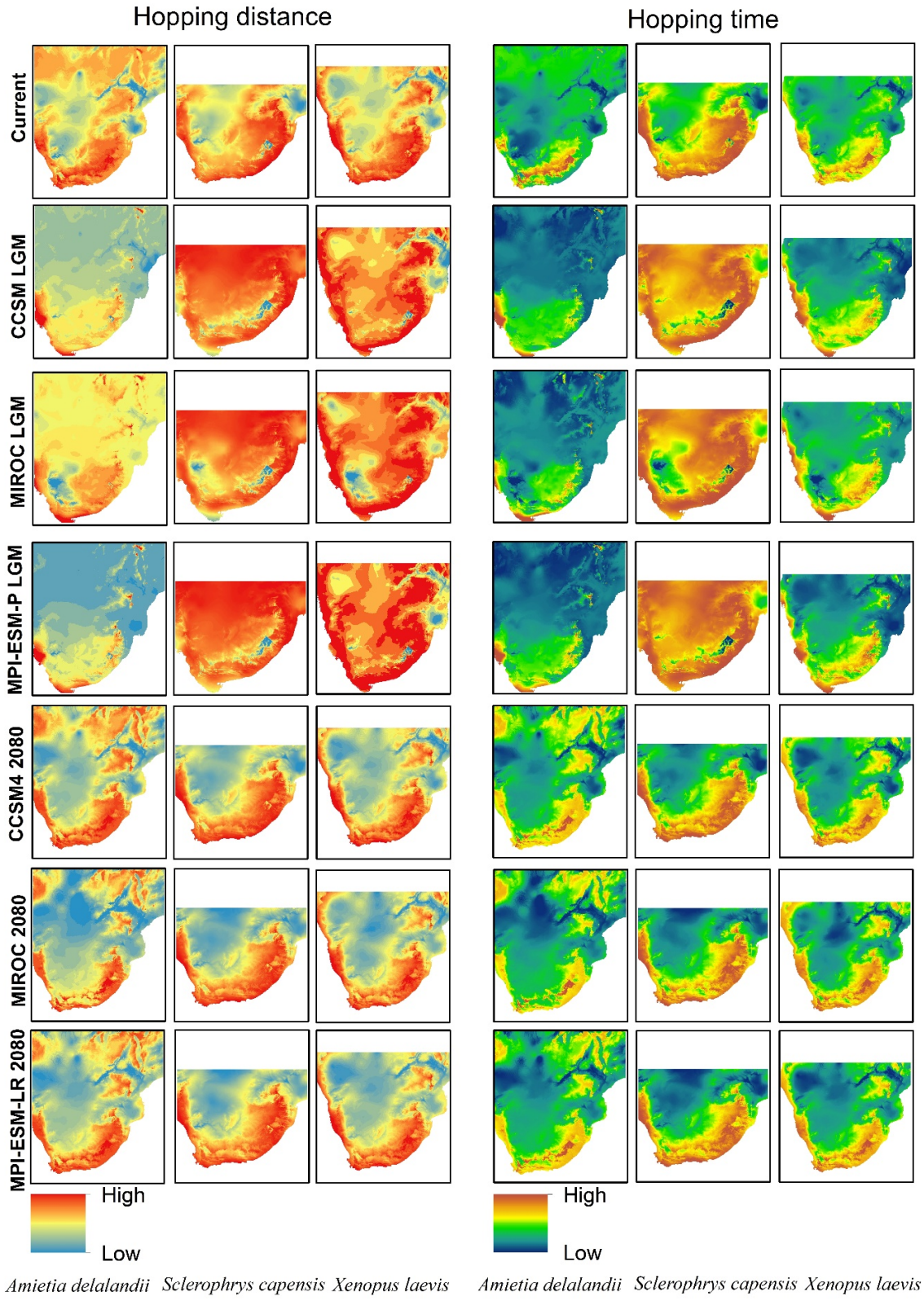
Supplementary information S6.2.

Supplementary information S6.3: Surface models constructed from hopping velocity and acceleration thermal performance measures.



Supplementary information S6.3.

Supplementary information S6.4: Surface models constructed from hopping distance and time (endurance) thermal performance measures.



Supplementary information S6.4.